HYDROLOGY, NUTRIENT REMOVAL, AND COST EFFECTIVENESS OF SMALL, EDGE-OF-FIELD TILE DRAINAGE TREATMENT WETLANDS

A Dissertation

SUBMITTED TO THE FACULTY OF THE

UNIVERSITY OF MINNESOTA

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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May 2019

Acknowledgements

I would first like to thank the members of my committee for providing the opportunity to conduct this research, for their confidence in me, and their guidance throughout the process. I am extremely grateful for Chris Lenhart's knowledge, guidance, and patience from the beginning. I owe a great debt to him for his willingness to invite me to work in his lab and for his direction in my research. There were some difficult stretches in my life during this work, and he graciously provided the space and patience I needed.

I also thank Heidi Peterson and others at the MDA for allowing me to participate in some amazing experiences with great committees and panels. I also thank Timothy LaPara, Joe Magner, Scott Matteson, Matthew Moore, and Tim Griffis for their expertise and guidance in their respective labs. I especially thank Darwin and Sandy Roberts for converting a portion of their property to the research wetland and providing easy access to the site whenever I arrived.

I thank Nikol Ross for collecting data at the wetland before I joined the project and introducing me to the site. Thanks also to Josh Gamble for harvesting and analyzing the biomass in the wetland, Sarah Winikoff for assisting in nutrient analysis of the mesocosm biomass, and Mark Greve for assisting in SWAT modeling. I also thank all the students in Chris Lenhart's lab and Andrew Sieben who contributed to data collection, construction, and maintenance at the wetland and in the lab.

I thank my family. Their commitment to supporting me throughout my life and education goes beyond the years of this work. They have always encouraged me no matter where life would lead me, but they had faith and taught me to work hard and use the gifts God has given me.

I thank Anselm House for allowing me to make myself feel at home in their study center. I am grateful for all the free coffee, snacks, and discussions on deep, intellectual topics. I thank all my roommates and friends who were patient even on the occasions when I needed to sacrifice time with them to work on my research. My small group and friends kept my faith strong by continually sharing theirs.

Last, but not least, I thank God for his grace. He gifted me with a passion for learning about how the world works and seeing beauty in it as well as a passion to care for others by caring for the environment around them.

Dedication

This work is dedicated to my late friends, Jamison, Kathryne, Ezra, Violet, and Calvin Pals. Their life and death inspired me to use my passions for the sake of others and to be willing to sacrifice for those who are being helped the least.

Abstract

Constructing treatment wetlands is a recommended practice for mediating nutrient pollution from non-point sources in the Mississippi River Basin. This research investigated the nitrogen and phosphorus removal effectiveness of a small, edge-of-field, constructed treatment wetland using field, laboratory, and modeling data. In the field, the wetland removed 67% (48-100%) of nitrate discharging from tile drainage but released soil legacy phosphorus from 2013 through 2016. Denitrification in the shallow groundwater and vegetation harvest were the greatest sinks for nitrogen and phosphorus, respectively. In the laboratory, three plant communities from the wetland (a wet prairie forb-dominant mix, a switchgrass and prairie cordgrass-dominant community, and a reed canary grass monoculture) were compared for nitrate removal. The wet prairie mix removed the most nitrate, and it had the lowest dissolved oxygen concentration and greatest ratio of denitrifying bacteria to total bacteria (*nosZ*:16S rRNA genes) – measured using a quantitative polymerase chain reaction (qPCR) – in its root zone. For the modeling component, the ACPF toolbox, the SWAT model, and a spreadsheet model were used to estimate the mass of nitrate-N removed from tile drainage if more edge-of-field wetlands were constructed in the Elm Creek HUC12 watershed. These smaller wetlands removed more nitrate-N per wetland area than larger wetlands (watersheds > 60 ha) but cost the same per mass removed if the small wetlands were designed to have a high saturated hydraulic conductivity. Results from this study suggest that edge-of-field wetlands can be more effective with a dual treatment of surface flow and shallow groundwater flow for nitrate removal and vegetation harvest for phosphorus removal.

However, reed canary grass invasion could potentially decrease the nitrate removal effectiveness. If the wetland soils have a high conductivity, the smaller, edge-of-field designs could be as cost effective as large treatment wetlands but remove less land from agricultural production.

This dissertation is composed of three individual chapters that will be published in peer reviewed scientific journals. The first chapter pertains to a field study that observed a small, edge-of-field tile drainage treatment wetland. This chapter will be submitted to *Ecological Engineering*. In the second chapter, the nitrate removal in three plant communities from the wetland was compared using mesocosms. Total bacteria and denitrifying bacterial populations in the root zones of these communities were also compared using qPCR. The work from this chapter was submitted to the *Journal of Environmental Quality* and is currently under review. The final chapter will be submitted to *Agriculture, Ecosystems & Environment*. This chapter compared the effectiveness of small, edge-of-field treatment wetlands with watersheds less than 60 ha to large treatment wetlands with watersheds greater than 60 ha. Multiple models were used to determine the best locations for each wetland in the Elm Creek watershed in southern Minnesota. Conclusions were drawn that small, edge-of-field wetlands are effective nutrient removal practices and can be improved with high saturated hydraulic conductivity, harvested vegetation, and diverse plant communities.

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General Introduction

Scope

According to the Clean Water Act (CWA) Section 303(d), each state is required to assess its waters to identify and list their impairments. These impairments are determined when a pollutant affects the ability of a lake, stream, or river to support the state's designated use of that water body (EPA 2013; MPCA 2008). In the 2018 Draft Impaired Waters List, the Minnesota Pollution Control Agency (MPCA) has 5,086 impairments listed from Minnesota surface water reaches across the state (2,452 303(d) TMDL listings). Of those reaches listed, 694 reaches designated for aquatic recreation or aquatic life were impaired from "nutrient/eutrophication biological indicators" (MPCA 2018).

Nitrogen is continuing to receive increased attention throughout the world. In Minnesota, the MPCA and other agencies have been focusing on this nutrient due to its harmful effects on people and aquatic life at high concentrations. It is also having a negative impact on the Gulf of Mexico where it is a limiting nutrient and thus causing algal blooms and hypoxic zones when added at high concentrations. Because many of Minnesota's waters discharge into the Mississippi River and eventually reach the Gulf, nitrogen reductions in Minnesota's fresh water plays a role in reducing the Gulf of Mexico's hypoxia zone (MPCA 2013). The 2008 Gulf of Mexico Hypoxia Action Plan calls for all states in the Mississippi River Basin to create nutrient reduction strategies. These reduction strategies are an attempt to decrease the load of nutrients contributing to the hypoxic zone in the Gulf of Mexico (Nutrient Task Force 2008).

In response to the hypoxia plan and the CWA, multiple states have developed nutrient reduction strategies. In Minnesota, the MPCA developed a reduction strategy in which it studied the conditions, trends, sources, and reductions of nitrogen across the state of Minnesota. As a result of this study, the MPCA concluded that on an average year, approximately 73% of the nitrogen in Minnesota's surface waters is from agricultural cropland, 9% is from wastewater treatment plants, and 18% is from an assortment of other sources (MPCA 2013). As nitrogen fertilizers are applied to row-crop fields, the dissolved form, mostly nitrate (NO_3^-), drains from the field in the subsurface drainage tiles (Follett and Delgado 2002). Due to an increasing use of subsurface tile drains and a high number of agricultural drainage ditches, the nitrogen in the drainage tiles or ditches is quickly discharged to nearby surface waters at concentrations oftentimes exceeding 10 mg L^{-1} nitrate, the drinking water standard for nitrate (EPA 2013; IDALS 2016b; MPCA 2013, 2014).

The other limiting nutrient of concern, phosphorus, is contributing to eutrophication in freshwater bodies (Schindler et al. 2008). Minnesota has set eutrophication standards that vary by the region of the state. Other states have similar standards in the Mississippi River Basin (IDALS 2016a; IEPA 2014; MPCA 2014). Most strategies for reducing the phosphorus loads target sources including streambank erosion, urban runoff, subsurface sewage treatment systems, and feedlots (MPCA 2014). Most of these sources are connected to surface runoff due to the conclusion that subsurface phosphorus transport is negligible. However, recent studies have been finding that phosphorus in surface waters has received significant contributions from subsurface agricultural tile drainage as well (King et al. 2015; Smith et al. 2015).

Nutrient reduction strategies list a variety of best management practices (BMPs) that can be implemented for treating water and preventing the release of nutrients into surface water. While reduction strategies are extensive, they rely on more research that will lead to the development of more efficient and effective practices. With the current technologies available, it is not possible for states to reach their nutrient reduction goals (IDALS 2016a; MPCA 2014). Continued research is necessary to improve the effectiveness of BMPs and assist in the achievement of the goals. This effort is of great importance in the Mississippi River Basin, but eutrophication is a problem worldwide. New improvements in technologies for reducing nutrient pollution will help clean water in more areas than the Mississippi River Basin. This project seeks to improve the effectiveness of one of the BMPs targeting these nutrients.

The BMP studied in this project is a constructed wetland used for removing nutrients discharged from subsurface agricultural tile drainage. Tiles typically drain directly into a drainage ditch or stream at concentrations often exceeding 10 mg L^{-1} nitrate-N, the current target limit for nitrate concentration in drinking water and cold water trout streams (EPA 2013; IDALS 2016a; MPCA 2014). Treatment wetlands could play a large role in the removal of nitrate reaching the gulf during the growing season if many of them are placed at the end of drainage tiles (Crumpton et al. 2006). A unique component of this wetland was that it was designed for a parallel study on harvesting native wet prairie vegetation from a treatment wetland for biofuels, so some of the species in the wetland was selected for that study (Current et al. 2016; Gamble et al. 2019).

Constructed wetlands have been used historically for treating human waste but have become more versatile in their treatment purposes. There are many designs which vary in efficiency for the variety of contaminants being treated. The design feature that has the greatest impact on treatment is the hydrology. Constructed wetlands can have a vertical flow, horizontal subsurface flow, or horizontal surface flow (Kadlec and Wallace 2008). Wetlands with horizontal flow have the greatest effectiveness at removing nitrate. However, vertical flow wetlands are better at removing particulate pollutants and ammonium (Vymazal 2007). Xue et al. (1999) concluded that denitrification is the dominant pathway for nitrate removal in treatment wetlands. As nitrate enters the wetland from tile drainage, denitrifying bacteria utilize nitrate as an electron acceptor once oxygen is depleted. In horizontal flow wetlands, the nitrate-rich water has a greater residence time in the soil layer with the greatest carbon source (Gardner and White 2010). As denitrifying microbes consume the carbon, nitrate is converted to nitrous oxide or nitrogen gas and removed from the water. In this way, a wetland can be a permanent sink for nitrogen.

Phosphorus, however, cannot be converted to a gas similarly to nitrogen. It discharges from tile drainage as either dissolved or particulate phosphorus (King et al. 2015). Phosphorus is then retained through sorption to soils, assimilation into plants or microbes, or accretion into soil layers. However, in each of these storages, phosphorus can eventually be returned to the water column through decomposition or desorption.

Phosphorus can be relatively secure in soil through sorption until the soil is saturated with phosphorus and bonding sites are no longer available, through accretion as long as layering continues to accumulate, and through assimilation until the organic matter decomposes (Johnston 1991; Kadlec 2016). Therefore, wetlands are not a permanent sink for phosphorus unless saturated soil is dredged or vegetation is consistently harvested. While wetlands can retain phosphorus for many years, more needs to be studied about how to improve phosphorus retention in wetlands through economically viable means.

Research Needs and Related Research

The processes involved in removing nitrogen and phosphorus in treatment wetlands have been thoroughly studied and published in research journals and textbooks (Kadlec and Wallace 2008; Mitsch and Gosselink 2000). While the mechanisms are well understood, more studies are needed that can improve the efficiency of those mechanisms and the implementation of the concepts. Many studies have been completed in the Midwest on the benefit of using wetlands to remove nutrients, especially nitrogen. Iowa State University has completed many of these studies and successfully constructed and restored wetlands throughout the state to reduce nitrate at the source (Crumpton 2001; Crumpton et al. 2006; Crumpton and Stenback 2016; Iovanna, Hyberg, and Crumpton 2008; Tomer et al. 2013). Other universities in the Midwest have also been productive in researching the role of wetlands in removing nitrogen and phosphorus (Fink and Mitsch 2004; Herr-Turoff and Zedler 2005; Hoagland et al. 2001; Kovacic et al. 2000; Mitsch, Zhang, et al. 2005; Mitsch and Fink 2001). Mitsch et al. (2005) and Crumpton (2006) have estimated approximately how many hectares of wetlands would be required in the Mississippi River Basin to reduce the load of nitrate in the basin by 30-40%. Their estimates vary from less than 1 million to over 2 million hectares. However, they both find treatment wetlands to be one of the most multi-beneficial BMPs available and one of the most effective at nutrient removal.

While there have been many studies on nutrient removal in wetlands, not many have been on small, edge-of-field tile drainage treatment wetlands that remove less than 0.25 ha of land out of agricultural production. One example of a small tile treatment design was thoroughly studied in Illinois. Kovacic et al. (2000), Xue et al. (1999), and Larson et al. (2000) concluded that the smaller design can be effective although it reduced less than 50% of the nitrate entering the wetland and it was not a major phosphorus sink. They also concluded that seepage and shallow groundwater treatment can play a role in improving denitrification. Saturated buffers installed in the Midwest have successfully utilized shallow groundwater flow to remove nitrate (Jaynes and Isenhart 2014; Utt, Jaynes, and Albertsen 2015), but it is still unknown whether infiltration should be encouraged in wetlands to utilize shallow groundwater reductions. Therefore, the impact of flow pathways needs to be studied more thoroughly. Furthermore, studies on small wetlands needs to be repeated to improve the confidence in the effectiveness of small tile drainage treatment wetlands for removing nutrients.

Beyond wetland design, there are still research gaps for understanding how vegetation and microbes affect nutrient reductions in these smaller wetlands. There is potential that highly productive plant species which tend to invade and dominate wetlands could influence the nutrient removal rate. Furthermore, macrophyte community composition could potentially influence denitrification (Gilbert 2004) and phosphorus removal (Johnston 1991; Vymazal 2007). Invasions from species such as *Typha x glauca* have correlated with increased denitrification (Lishawa et al. 2014). However, invasions from species such as *Phalaris arundinacea* may not increase nutrient removal despite its highly productive physiology (David et al. 1997; Herr-Turoff and Zedler 2005; Swanson et al. 2017). With the high probability of small wetlands' plant communities shifting, especially if resources are unavailable to extensively manage the vegetation, there needs to be a greater understanding of what will happen to the nutrient removal following an invasion.

Another feature of these wetlands that is not well understood is the relationship between denitrifying bacterial populations and vegetation. There seems to be a relationship between denitrifying bacterial populations and denitrification rates in some locations (Baxter et al. 2012), but there are studies where locations of greatest denitrification potential were not the same as the locations with greatest denitrifying microbial populations (Kozarek et al. 2017; Wang et al. 2013). The variability in these relationships means more studies need to be conducted to understand what role denitrifier population abundance may play in denitrification, especially in smaller wetlands.

Finally, many studies are conducted to measure the performance of BMPs in the lab or in the field, but not all are followed by modeling studies which estimate the potential application and implementation of that BMP. Some BMP evaluations are being modeled at a watershed scale across the Midwest (Folle et al. 2007; Gassman, Sadeghi, and Srinivasan 2014; Tomer et al. 2015). One study modeled the siting and potential nitrogen reductions of treatment wetlands in an agricultural watershed in Illinois (Tomer et al. 2013). Other designs and updated technologies need to be modeled to estimate watershed-scale reductions of nutrients and costs.

Objectives

One goal of this dissertation research is to better understand aspects of small, edge-of-field constructed tile drainage treatment wetlands which will assist in the improvement of their effectiveness. A second goal is to understand how they can aid in reducing nutrient loads from tile drainage across watersheds. More specifically, this research will seek to 1) understand the effect of various flow pathways on nutrient reductions; 2) determine the role of plants and microbes on nutrient removal; and 3) compare the effectiveness and costs of small wetlands to larger tile treatment wetlands that have proven to be successful in Iowa (Crumpton and Stenback 2016). Each study had its own objectives pertaining to the same treatment wetland.

To pursue these goals, dissertation research comprised the following three major study objectives:

1) Evaluate the nitrate and phosphorus reductions in surface flow and shallow groundwater flow by measuring nitrate-N and total phosphorus across the surface and shallow groundwater flow paths of a treatment wetland and determine the impact of harvesting vegetation on the nutrient removal effectiveness of the wetland.

2) Compare nitrate reductions and denitrifying microbial populations in wet prairie plant communities and a reed canary grass monoculture to determine the impact of reed canary grass invasion on the effectiveness of nitrogen reduction through a mesocosm and microbial DNA study.
3) Model the nitrate removal and cost effectiveness of small, edge-of-field treatment wetlands compared to tile treatment wetlands with drainage areas larger than 60 ha if they were constructed in the Elm Creek HUC12 watershed.

Dissertation Organization

This dissertation contains a general introduction (this chapter: chapter 1), three research chapters (chapters 2-4), a list of references, and three appendixes associated with each respective research chapter. One research chapter is devoted to each of the three goals listed above. Chapter 2 is titled, *Reduction of nutrient loads from agricultural subsurface drainage water in a small, edge-of-field constructed treatment wetland*. Chapter 3 is titled, *Nitrogen reductions and microbial populations in* Phalaris arundinacea *and wet prairie mix wetland mesocosms*. Chapter 4 is titled, *Modeling the applicability of edge-of-field treatment wetlands to reduce nitrate loads in the Elm Creek, Minnesota watershed*. Each chapter has been or will be submitted to peer-reviewed journals. The first three years of observations in chapter 2 were published in *Water* in 2016, but a more detailed analysis of the shallow groundwater along with the fourth year of observations will be included in a separate publication.

Authors of the research include Brad Gordon, Christian Lenhart, Joshua Gamble, Dean Current, Nikol Ross, Lydia Herring, John Nieber, Heidi Peterson, Joe Magner and Timothy LaPara. Mr. Gordon designed and implemented the experiments, collected and analyzed the data, and wrote the chapters. Christian Lenhart, John Nieber, Lydia Herring, and Nikol Ross designed the wetland and provided oversight during its construction. Drs. Lenhart, Current, Nieber, and Peterson provided oversight and input for the research. Dr. Magner provided input and funding for the subsurface flow isotope analysis in Chapter 2. Dr. LaPara provided input and oversight for the qPCR methods and data analysis in Chapter 3.

Chapter 1: Reduction of nutrient loads from agricultural subsurface drainage water in a small, edge-of-field constructed treatment wetland

Abstract

Constructed treatment wetlands are a common practice for filtering nitrogen from agricultural tile drainage in the Midwest. Wetland size recommendations vary, but little is known about the effectiveness of wetlands with a pooled area as small as 0.1 hectare. A small, edge-of-field wetland treating tile drainage from a 10.1-ha row-crop field was constructed in 2013 adjacent to Elm Creek near Granada, MN. The water, nitrate, and phosphorus budgets were determined from 2013 to 2016. The wetland received 55,268 m^3, 738 kg nitrate-nitrogen (NO_3-N), and 5.2 kg total phosphorus (TP) over the four years of this study. It removed 67% of the input nitrate but released 1.6 times more TP than the input load. The majority of nitrate was removed in the shallow groundwater flow after it infiltrated from the surface. Approximately 0.35 kg-P yr^{-1} was removed by harvesting the vegetation in the wetland, but as much as 0.98 kg TP yr^{-1} was released from the soil. By the end of the study, most of the soil phosphorus was released from the soil, making the wetland more likely to reduce phosphorus after 4-6 years following construction.

Introduction

Restored and constructed treatment wetlands have been used often to treat wastewater and agricultural runoff. There are various types of wetland designs from surface flow to subsurface flow wetlands to treat wastewater containing excess nutrients, biosolids, sediment, and other pollutants (Kadlec and Wallace 2008, Miller et al. 2012). Many have been constructed across North America. The Iowa Conservation Reserve Enhancement Program (CREP) has been making significant progress in restoring large wetlands for the reduction of nitrogen in agricultural watersheds. As of the 2016 report, 83 wetlands have been restored through that program since 2001. These wetlands total 3.1 km^2 (Crumpton and Stenback 2016). However, they are still too few to address the issues caused by agricultural runoff in the upper Midwest. According to Mitsch et al. (2001; 2005), approximately 22,000 km^2 of wetlands need to be restored or created in the Mississippi River basin to reduce the load of nitrogen to the Gulf of Mexico by 40%. This estimate does not include other best management practices (BMPs), but it provides a perspective that many more acres of wetlands, in collaboration with other practices, are still required to reduce the load of nitrogen in the Mississippi River. In *The Agricultural BMP Handbook for Minnesota*, surface flow wetlands are recommended for this purpose due to their greater potential for nitrogen reductions as compared to subsurface flow wetlands which are considered to be more effective for sediment, ammonium, and phosphorus reductions (Lenhart et al. 2017; Mitsch and Jørgensen 2004; Vymazal 2007). In addition to water quality benefits, wetlands also improve biodiversity, fish and wildlife habitat, water storage, and recreation.

As wetlands are being constructed to treat agricultural runoff, decisionmakers and practitioners need to know more information about which designs work best for nutrient reductions. For example, a deep-water wetland may improve the ability of the wetland to store more water and improve anaerobic conditions, but some are too deep for many wetland plant species. Many of these plants would improve the effectiveness of the wetland at removing nitrogen in the water column, and preventing wind-driven re-suspension of sediment and phosphorus (Fransen 2012). Therefore, a shallow wetland may be better for biodiversity and nutrient retention (Hansson et al. 2005; Vymazal 2007). The National Engineering Handbook recommends sealing the bottom of wetlands in order to prevent infiltration loss (USDA NRCS 2009). However, more needs to be explored about the positive role of infiltration into shallow groundwater for nitrate reduction in treatment wetlands alongside the impacts of fluctuations in wet and dry periods during the growing season (Brauer et al. 2015). In some locations, groundwater contamination is a concern, but there have been studies showing the potential for nitrate reductions in the shallow groundwater of wetlands designed primarily for surface flow (Brauer et al. 2015; Larson et al. 2000). However, the effectiveness of these treatment wetlands needs to be better understood (Brauer et al. 2015; Brodie 1989).

Although treatment wetlands are not a common phosphorus removal practice in state nutrient reduction strategies in the Upper Mississippi River Basin, there is still potential for these systems to function as phosphorus removal practices. In Denmark, there have been multiple studies with positive phosphorus removal results in treatment wetlands that have led to their inclusion for phosphorus reduction in governmental action plans (Hoffmann et al. 2006; Hoffmann, Kronvang, and Audet 2011; Kronvang et al. 2007). Some studies in the Midwest also revealed the potential for phosphorus reductions in wetlands (Huang, Mitsch, and Johnson 2011; Mitsch 1992; Mitsch et al. 1995). Results from a literature review by Leah Smith at the University of Minnesota, combined with values provided by Lenhart et al. (2017) and the Minnesota Stormwater Manual (MPCA 2016b), estimate that constructed treatment wetlands could reduce total phosphorus by an average of 30% (Appendix 1, Table 29).

Wetlands can remove phosphorus, but they have the potential to release it back into the water column during anaerobic conditions (Fransen 2012; Mitsch and Gosselink 2000). While the historic assumption has been that most phosphorus is attached to sediment and transported in surface runoff, thus making phosphorus in most tile drains minimal, there are regions where phosphorus concentrations in tile drainage can be high (Christianson et al. 2016; Smith et al. 2015). Unfortunately, phosphorus cannot be converted to a gas similarly to nitrogen, so phosphorus must either settle in sediment or be removed through soil dredging or vegetation harvest. One study in Poland evaluated this concept and estimated approximately 14 kg-P could be removed for every hectare of willows harvested from the wetland (Skłodowski et al. 2014). In Canada, harvested cattails removed an estimated 20-60 kg-P ha^{-1} each year (Grosshans et al. 2014). Another study of haying wet meadows and fens revealed that 2-23 kg-P ha^{-1} could be removed each year (Venterink et al. 2002). Therefore, depending on the load entering the treatment wetland, phosphorus removal could be improved by harvesting the vegetation.

The objective of this study was to evaluate the effectiveness of a small, edge-of-field agricultural treatment wetland and the impact of infiltration into shallow groundwater and plant harvest for nutrient removal. Nitrate/nitrite-N, total phosphorus, and soluble orthophosphorus were measured across the surface flow path of a treatment wetland and within shallow wells. The project was originally designed to evaluate only surface reductions of nutrients, but a much larger portion of the water in the wetland infiltrated into the shallow groundwater than expected in the first two years. Therefore, the objectives evolved to include shallow groundwater evaluations as well. The hypothesis was that shallow groundwater treatment and plant harvest in this wetland would improve its overall effectiveness.

Materials and Methods

Site Description

The small, edge-of-field treatment wetland was in Martin County 6.9 km north of Granada, MN (43.756562 N, 94.343852 W; Figure 1). The wetland was designed in 2012 and constructed in the spring of 2013 (Karlheim 2012; Lenhart et al. 2016). Approximately 10.1 ha of subsurface tile drainage was rerouted to flow into the wetland (Figure 2). The western half of the wetland had been farmed for over 50 years while the eastern half remained as a hardwood forest during that time (Figure 3). The wetland was placed in the historic floodplain of Elm Creek. This area is part of the Des Moines Lobe glacial till plain covering much of southern Minnesota and northern Iowa. The dominant soil types in this floodplain include Coland clay loam and Spillville loam. The Coland clay is a mesic Cumulic Endoaquoll soil with 0.9-1.2 meters of clay loam on the surface and sandy soil underneath. The dominant soil type on the surrounding uplands is the Clarion-Storden complex, a well-drained loam on 6-12 percent slopes (Matzdorf 1989).

The property is a research and demonstration site for several local and state agencies in partnership with the University of Minnesota and non-profits. Tile drainage entering the wetland previously discharged directly into Elm Creek. This creek flows to the east into the Blue Earth River. This river connects with the Minnesota River which eventually flows into the Mississippi River near St. Paul, MN (Figure 1) and then toward the Gulf of Mexico. The land use of the Elm Creek watershed (Hydrologic Unit Code [HUC] 12) is dominated by corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) row crops. The 10.1-ha watershed of the wetland rotated between corn and soybeans each year of the study. There was a cereal rye (*Secale cereale* L.) cover crop on the northern half of the watershed in the winter of 2014-2015 and a radish (*Raphanus sativus* L.) cover crop on the southern half of the watershed in the winters of 2014-2015 and 2015-2016.

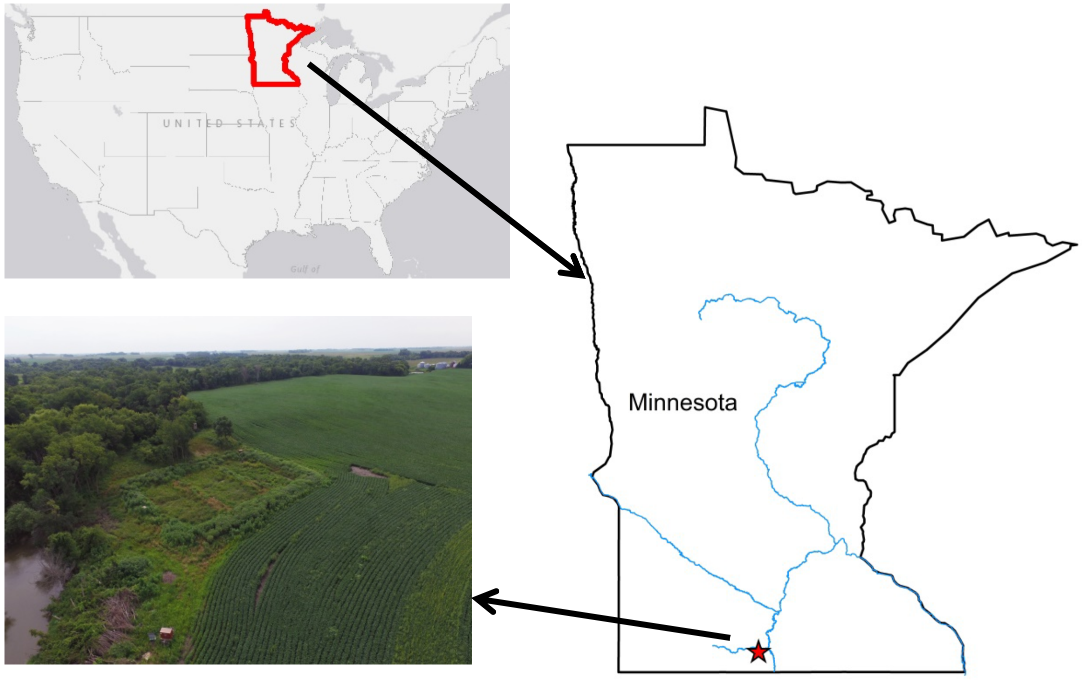

Figure 1. Location of the treatment wetland adjacent to Elm Creek which eventually flows into the Mississippi River along Minnesota's southeast border.

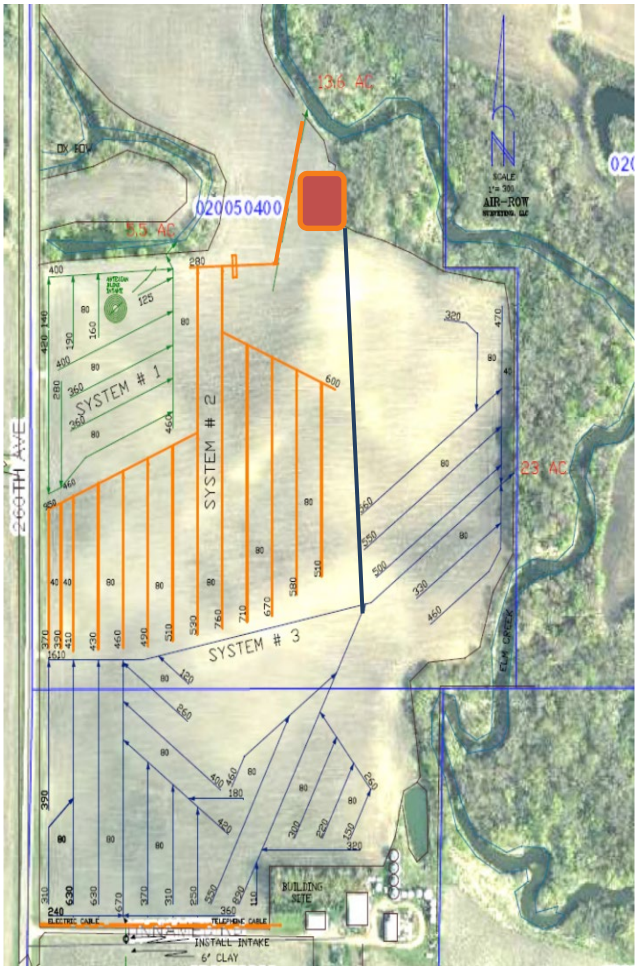

Figure 2. Sub-surface tile drainage network showing the areas draining to the treatment wetland (shown as orange rectangle). System 3 and another system south of the driveway drain into the wetland.

Figure 3. Aerial image of the portion of Darwin and Sandy Robert's property containing the wetland's 10.1-ha watershed. The wetland and its buffer cover 0.22 ha along the northern edge of the tile drained row crop field north of Granada, MN. The wetland is within the yellow rectangle, and the Roberts' driveway runs horizontally across the middle of the image.

Tile drainage in the wetland's watershed averages 24-m spacing with 0.9-1.2-m depth (Figure 2). Tiles have a greater spacing in the northern portion of the drainage area than in the southern portion. Tile discharging into the wetland is a 15.2-cm corrugated plastic pipe. The farmer also has an Agri Drain control structure in the middle of the watershed where he can hold back water or open gates depending on the saturation of the soil. The farmer's driveway divides the watershed into two sections (Figure 3). The southern section drains into the wetland, but the northern portion is separated into three drainage areas. The northwestern section is approximately 7.3 ha and drains to a woodchip bioreactor 50 meters to the northwest of the wetland. The eastern section is approximately 5.3 ha and drains to a woodchip bioreactor on the eastern edge of the field. The remaining 4 ha of that field, the southern portion, drain toward the wetland. This drainage combines with the 6.1 ha south of the driveway. During high flow events, the farmer has the option to divert excess water from the 6.1-ha southern field toward the eastern bioreactor where it bypasses the bioreactor and flows directly to the creek.

The county in which the treatment wetland is located, Martin County, historically receives 76-81 cm of precipitation each year. It is one of the warmest counties in the state of Minnesota. Its average annual temperature is approximately 8 ℃ (University of Minnesota 2017).

Wetland Design

The wetland is a 3-cell design with the first cell acting as a forebay and the subsequent cells designed to increase the residence time of the water (Figure 4). Due to this wetland receiving only tile drainage and no overland flow, sedimentation did not play a large role in the forebay. However, the first cell was the most active cell. Each cell was

13.7 m by 26.7 m. Berms separated each cell and surrounded the entire wetland. The berms separating the cells were 0.5 m in height and the perimeter berm was 1.4 m higher than the wetland floor. With the berms included, the total wetland area was 0.22 ha. However, only 0.11 ha were frequently inundated.

A unique component of this wetland design was the multi-functional purpose of the project. While the wetland was designed to study how well it could remove nutrients, it was also designed to study potential biofuel crops. The seed mix chosen for this wetland therefore needed to serve both research projects. The mix was intended to be diverse, needed to grow well in wet and dry conditions, and would grow well under a harvest regime. Each cell was seeded with a wet prairie mix (Appendix 1; Table 30). The first cell had a low-diversity mix (12 species), the second cell had a medium-diversity mix (20 species), and the third cell had a high-diversity mix (32 species). After seeding the wetland, a large rain event resulted in flash flooding which washed much of the seed from the first cell. That cell was reseeded in August of the first year with a 23-species native mixture. In September 2014, reed canary grass was spot sprayed with a.i. glyphosate [2-[(phosphonomethyl)amino]acetic acid]. In 2015, reed canary grass was sprayed twice with a mix of 18% a.i. glyphosate [2-[(phosphonomethyl)amino]acetic acid] and 0.73% a.i. diquat dobromide [1,1'-Ethylene-2,2'-bipyridyldiylium dibromide], and tree saplings growing in the wetland were cut down. When the vegetation was harvested to assess its potential as a native mix biofuel as part of the other study, the mass of nitrogen and phosphorus removed through harvesting were also measured.

Data Collection and Calculations

General Hydrology

The following equation was used as a framework for comparing different components of the water budget in the entire wetland:

Equation 1:

$$Q_{in} + P + GW_{in} = Q_{out} + ET + GW_{out}$$

Q_{in} = subsurface tile drainage entering the wetland

P = precipitation in wetland area

GW_{in} = groundwater discharging into the wetland

Q_{out} = water discharging from wetland outlet

ET = evapotranspiration

GW_{out} = groundwater recharge through infiltration leaving the wetland

The water budget components were estimated using a variety of tools and equipment. The berms surrounding the wetland prevented any overland flow from entering the wetland, so overland flow was not included in the equation. The precipitation was measured with HOBO®, Davis®, and ISCO® rain gauges. The rainfall amounts were also doublechecked using data from the University of Minnesota Climatology Working Group and the Fairmont, MN, airport weather station. The measured rainfall was multiplied by the catchment area of each treatment cell in the wetland (0.04 ha each) to calculate the volume. The potential evapotranspiration (ET) was estimated using the Hamon and Thornthwaite potential ET equations (Lu et al. 2005, Federer 1996) from temperatures collected on an EasyLog® USB temperature logger.

Surface Hydrology

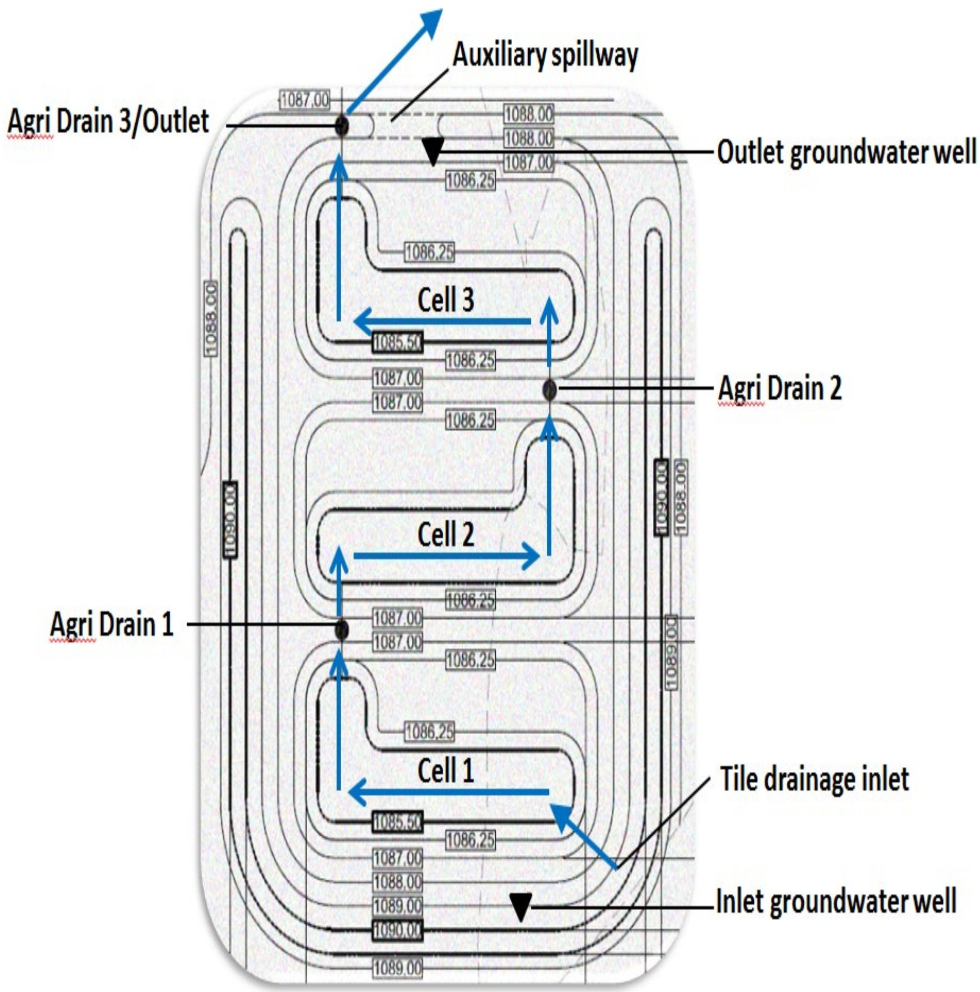

Figure 4. The design of the treatment wetland, and a depiction of the surface water flow through the wetland from south to north (blue arrows).

Tile inflow and outflow were measured using area velocity probes and pressure transducers. ISCO® Area Velocity Flow Loggers were installed at the inlet and outlet to record the level and velocity of water flowing into and out of the wetland every 15 minutes. The level measurements of these instruments had an accuracy estimate of ±0.003 m, and the velocity had an accuracy of ±0.03 m s^{-1}. Equations from Bengtson (2012) were used to estimate the area of the partially full pipe to multiply by the velocity measured.

Solinst Leveloggers® (pressure transducers), which had an estimated accuracy of ±0.05%, were placed in each Agri Drain box to estimate water flowing from each cell using flat board weir equations (Equation 2 and Equation 3; Chun and Cooke 2008). The Leveloggers® recorded the level every 10 minutes. A Levelogger® was also placed in the inlet and outlet Agri Drain control. These monitoring devices were more accurate than the area velocity loggers. Infiltration into the shallow groundwater was then calculated once all the other variables in the above water budget equation were determined.

Equation 2:

$$\mathrm{Q} = 0.027(\mathrm{WH})^{1.2} \qquad \mathrm{H} > 0.44\mathrm{W}$$

Equation 3:

$$\mathrm{Q} = 0.020(\mathrm{W} - 0.427\mathrm{H})\mathrm{H}^{1.48} \quad \mathrm{H} \leq 0.44\mathrm{W}$$

Q= Flow rate (L sec^{-1})

H = Height of water above the board (cm)

W = Width of the board (cm)

In 2015, the accuracy of measuring flow in the control structures was improved by replacing the top board in each structure with a v-notch weir. Measuring the height of water flowing over a smaller surface in the v-notch improved readings during low flow. The weirs were calibrated in the laboratory to the following curve by Minnesota Department of Agriculture staff to calculate the flow rate (Equation 4; Rassmussen and Matteson 2017):

Equation 4:

$$Q = 0.9833x^{2.0801}$$

x = stage of water in feet

Q = flow rate ($ft^3\ sec^{-1}$)

The flow rate was converted to $m^3\ min^{-1}$ and multiplied by 10 minutes to calculate the volume. The error ranges were estimated by using the accuracy value of each instrument. The hydraulic loading rate was then calculated by dividing the volume flowing into the wetland each year by the surface area of the wetland.

Techniques for Estimating Flow during Data Gaps

In 2014, inflow from the tile drain was not measured from March 23rd to June 5th due to the instruments malfunctioning. The missing flow was estimated by using the Q:P ratio (watershed discharge : precipitation ratio) of 2016 when accurate flow was measured (Fransen 2012). The agricultural practices in 2016 better matched those of

2014 than 2015's practices did. The cover crops may have had a significant impact on discharge in 2015 compared to 2014 and 2016. The Q:P ratio for 2016 was 0.36. The Q:P ratio was multiplied by the rainfall for each day of rainfall. The error ranges for these estimates were much larger than those from the instruments. They were estimated by recalculating the inflow to the wetland using ratios of 0.1 and 0.5 because these are the ratio ranges estimated by Fransen (2012) in the same region of Minnesota.

Water volumes discharging from the wetland's surface outlet were also missing from March 23rd to June 5th due to physical damage to the equipment. Due to the missing flow data from the outlet in 2014, flow collected from the outlet was compared to flow from Agri Drain 2 at times when both were measured. Two equations (Equation 5 and Equation 6) were developed in order to estimate missing flow in the outlet from the flow calculated during the corresponding dates in Agri Drain 2.

Equation 5:

$$Q_{out} = 0.08316\ln(Q_{AD2}) + 0.0301 \qquad \text{if } Q_{AD2} < 0.00722 \text{ m}^3 \text{ sec}^{-1},\ R^2=0.92$$

Equation 6:

$$Q_{out} = 0.02176(Q_{AD2}) - 0.07386 \qquad \text{if } Q_{AD2} \geq 0.00722 \text{ m}^3 \text{ sec}^{-1},\ R^2=0.96$$

Q_{AD2} = Flow rate through Agri Drain 2 (m^3 sec^{-1})

Q_{out} = Flow rate through the wetland surface outlet (m^3 sec^{-1})

Determining Flooding or Backflow Dates

During the four-year study of the treatment wetland, there were multiple occasions when Elm Creek flooded over its banks and reached the berms of the wetland. While flood water never flowed over the berms into the wetland, there were periods when flood water blocked the discharge from the surface outlet pipe. This made the flow rate equations inaccurate at those times. Therefore, any water level readings when water was blocked from flooding needed to be eliminated from the dataset. The times of these flooding occurrences were determined when the height of water was higher in the third cell's outlet than in its inlet structure.

Infiltration

Infiltration in this study was defined as water slowly passing through the soil from the surface of the wetland into the shallow groundwater. The water table was within one meter of the surface, and the shallow groundwater flowed laterally toward Elm Creek. On October 10, 2014, a 10.2-cm diameter infiltrometer was pressed into the soil at two locations in cell 1. The vertical saturated hydraulic conductivity (Ksat) was measured using similar methods to those in Mohanty (1994). The change in head within the infiltrometer was measured four times over the course of 30 minutes.

The measured Ksat values were compared to the modeled Ksat value from the spreadsheet model used by Lenhart et al. (2016) to design this wetland. The model was created in Microsoft® Excel to calculate the daily water budget and nitrogen removal based on wetland size, weir size, daily precipitation, tile discharge, tile nitrate concentration, evapotranspiration, vertical saturated hydraulic conductivity, and the nitrate reaction coefficient. The spreadsheet model used Equation 7 below to determine the daily flow out of the wetland (Karlheim 2012). It also calculated the daily volume infiltrating into the shallow groundwater based on a manually entered vertical Ksat. Field-measured water budget inputs and potential evapotranspiration were manually loaded into the model, but the Ksat was adjusted until the surface discharge matched what was measured each year.

Equation 7:

$$Q_o = C_E * W_w (H_o - H_w)^{1.5}$$

Q_o = outflow rate (m^3 day^{-1})

C_E = weir discharge coefficient (m^3 day^{-1}) ($m^{2.5}$)

W_w = width of weir (m)

H_o = water surface elevation at wetland outlet (m)

H_w = weir crest elevation (m)

Shallow Groundwater Flow

Shallow groundwater wells were installed each year of the study. By the start of the fourth year, there were eleven wells and four piezometers (Figure 6). The four piezometers had perforations of 30 cm at their base and were installed to determine if there was an upward or downward gradient in the shallow groundwater. The transects of wells were arranged to calculate gradients of shallow groundwater flow as well as reductions of nitrogen and phosphorus as water infiltrated into the shallow groundwater. Most of the wells were installed slightly below the clay topsoil. The topsoil was approximately 1.0 - 1.5 meters thick and was a clay to clay loam. Beneath the top layer, permeability increased in a sandy loam layer that was approximately 1 – 2 meters thick with a little gravel dispersed throughout the layer. The water table was observed within the clay loam or the topsoil layers throughout the study (Figure 5).

The horizontal saturated hydraulic conductivity was measured in the sandy loam soil below the surface of the wetland by using falling slug tests on November 16, 2016 in the four piezometers. Water level data were added to spreadsheets prepared by Shapiro and Greene (Halford and Kuniansky 2002). These spreadsheets calculated the horizontal saturated hydraulic conductivity (Ksat; m day^{-1}).

Water levels in wells were triangulated from periods when there was no surface water in 2015 and 2016 to determine the flow direction of shallow groundwater through the wetland (Figure 6). The Darcy Flux between two wells was calculated using the following equation:

Equation 8:

$$\frac{dh}{dl} K_{sat} = D$$

dh = difference in water levels

dl = distance between the two wells

K_{sat} = saturated hydraulic conductivity (m day^{-1})

D = Darcy Flux (m day^{-1})

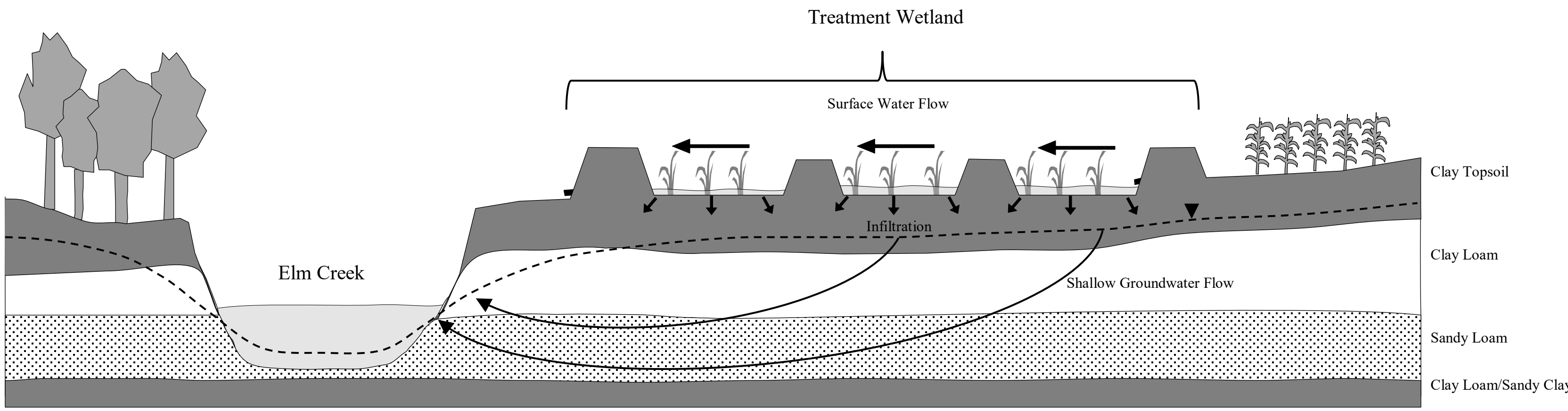

Figure 5. Schematic of the surface water, infiltration, and shallow groundwater flow at the wetland. Each soil layer was approximately 1 meter thick.

The horizontal velocity and residence of shallow groundwater between two wells were calculated with the following equations:

Equation 9:

$$V = \frac{D}{P}$$

V = average groundwater velocity (m day^{-1})

D = Darcy Flux (m day^{-1})

P = effective porosity; 0.06 m^3 m^{-3} [(Domenico and Schwartz 1990; Morris and Johnson 1967); for clay soil]

Equation 10:

$$T = \frac{dl}{V}$$

T = residence time

dl = distance between the two wells

V = average groundwater velocity (m day^{-1})

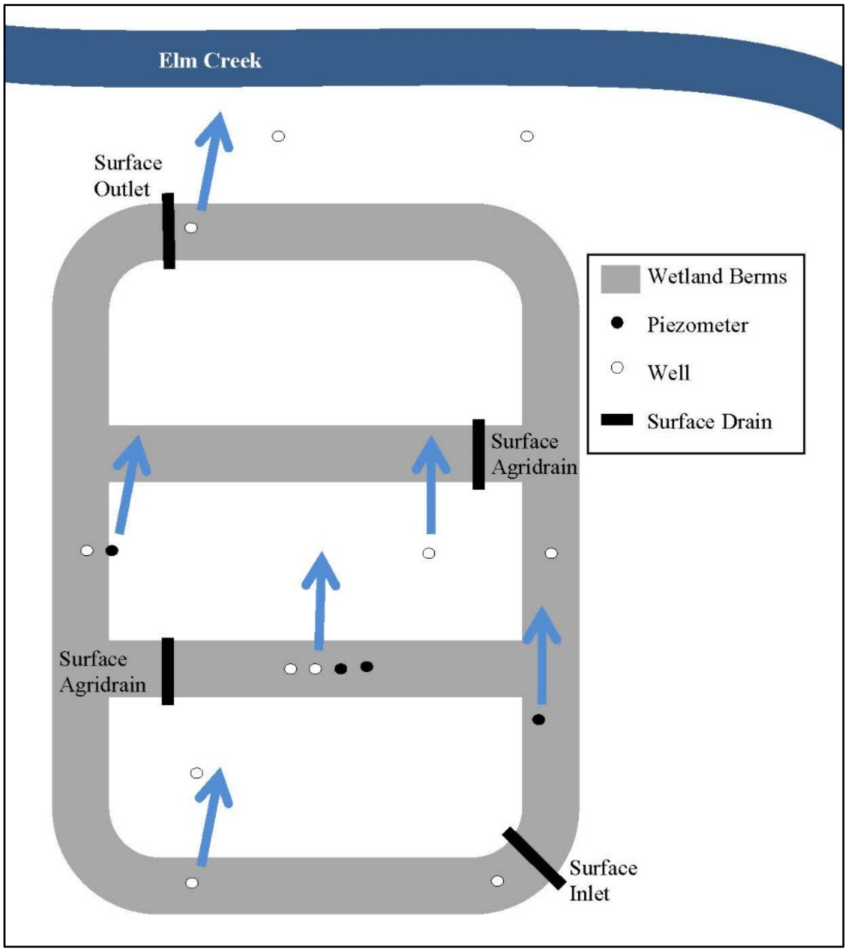

Figure 6. Schematic of the wells, piezometers, and shallow groundwater flow direction through the wetland.

Stable isotope tracers such as deuterium (δD) and oxygen-18 ($\delta^{18}O$); ion tracers such as calcium, silica, or sodium; and specific conductance (SC), among other tracers, have been used to quantify surface runoff versus groundwater discharge in streams for the past 50 years (Miller et al. 2014). Both δD and $\delta^{18}O$ and specific conductivity (SC) were used to distinguish deep groundwater potentially mixing with infiltrated surface water within the shallow groundwater beneath the wetland. For the stable isotopes, grab samples were collected from the wells on April 2, June 12, June 19, and October 24 of

2015. Isotope ratios were measured in the lab using isotope ratio infrared spectroscopy (IRIS). Due to the lighter isotopes evaporating before heavier isotopes, groundwater typically will have a higher ratio of heavy to light isotopes than surface water.

Specific conductivity was measured every 30 minutes using a HOBO® freshwater conductivity logger in the inlet tile drain in 2015 and in both the inlet and one of the wells next to cell 1 in 2016. Hand measurements were taken on four dates in the wells and the surface water using a YSI® Professional Plus Multiparameter Instrument. SC was also measured throughout the site in 2012 before the wetland was constructed. These measurements included three deep groundwater samples from the southern edge of the property to establish a baseline SC for the aquifer beneath the study site.

Residence Time

The average residence time on the surface of the wetland was calculated using the volume of water in the wetland and the measured flow rate (Equation 11).

Equation 11:

$$\tau_{avg} = \frac{1}{T}\int_{0}^{T}\frac{V(t)}{Q(t)}dt$$

τ_{avg} = Average residence time (days)

T = Total duration of measurements (days)

$V(t)$ = Volume in the wetland at time, t (m^3)

$Q(t)$ = Flow rate at outlet at time, t (m^3 day^{-1})

For periods when there were no measurements in Agri Drains 1 and 2, the distance from the north berm of cell 1 was used due to most of the water infiltrating from cell 1. Residence time of shallow groundwater was calculated using the following equation:

Equation 12:

$$\tau_{gw} = \left(\frac{D_{C1}}{r}\frac{V_{C1}}{V}\right) + \left(\frac{D_{C2}}{r}\frac{V_{C2}}{V}\right) + \left(\frac{D_{C3}}{r}\frac{V_{C3}}{V}\right)$$

τ_{gw} = Average residence time of infiltrated water (days)

r = Average groundwater velocity (m day^{-1})

D = Distance from center of that cell to creek (m)

V_c = Volume infiltrating from within that cell (m^3)

V = Volume infiltrating from within all cells (m^3)

Nutrient Samples

Grab samples were taken throughout each growing season from the inlet, outlet, Agri Drains, and wells. These were submitted to an EPA-certified testing laboratory to be measured for nitrate/nitrite-N, total phosphorus, and soluble orthophosphorus during each of the four years. Nitrogen was also occasionally measured on site using a Hach® Nitratax sc, UV Nitrate sensor in the third and fourth years.

Samples for nitrate/nitrite-N concentration measurements were taken from each well by using a peristaltic pump or hand pump to draw the sample. The shallow groundwater consistently flowed in the same direction when the surface of the wetland was dry. Therefore, samples during dry conditions were used as baseline samples for estimating denitrification reaction coefficients in shallow groundwater. Two wells in line with the flow direction were used to estimate the denitrification reaction coefficient with the following equation:

Equation 13:

$$K = -\frac{1}{T}\log(\frac{C_2}{C_1})$$

K = reaction coefficient for nitrate removal (day^{-1})

T = residence time between wells (days)

C_2 = nitrate concentration in downgradient well (mg L$^{-1)}$

C_1 = nitrate concentration in upgradient well (mg L^{-1})

The K reaction coefficient was calculated on three different dates when the wetland was dry and was inserted into the spreadsheet from Lenhart et al. (2016). The spreadsheet calculated the daily water budget which included surface water infiltrating into the shallow groundwater. It used daily concentrations of nitrate on the surface, daily volumes of water infiltrating into the shallow groundwater, residence time in the shallow groundwater, and the K reaction coefficient of shallow groundwater to estimate how much nitrate was removed in the shallow groundwater.

Total phosphorus and soluble orthophosphorus were sampled from the wells on four dates in 2015 and 2016. There was not a consistent trend in how these concentrations changed as the shallow groundwater flowed toward the creek, so changes could not be estimated using similar methods to nitrate. Because phosphorus is likely to exchange between dissolved forms and particulate-bound forms, soil samples were also collected in 2012, 2014, and 2018. In 2012, six total samples were collected before the wetland was constructed. Three were from the top 15 cm and three were from 15-30 cm. In 2014, six total samples were collected from the top 30 cm. In 2018, 28 total samples were collected from the top 2 m of soil. All the soil samples were submitted to the Research Analytical Laboratory (RAL) at the University of Minnesota. Because the soil had a pH over 7.5, phosphorus was extracted using 0.5 M $NaHCO_3$ at a pH of 8.5 and determined by the molybdate-blue method using ascorbic acid as a reductant (Frank, Beegle, and Denning 1998). A bulk density of 1.3 g cm^{-1} was multiplied by the volume of soil in the top 0.5 meters of the wetland and the results of the soil phosphorus test to estimate how much phosphorus was adsorbed to the soil during each soil sampling period. These measurements were then used to estimate how much the soil phosphorus changed over the first six years of the wetland.

Nutrient removal through vegetation assimilation was estimated in the wetland as part of the biofuel assessment project at the same time as this study (Gamble et al. 2019). In 2014, all vegetation was harvested throughout the wetland. In 2013 and 2015, only two 1-m^2 areas were harvested from each wetland cell. In 2013-2015, vegetation was harvested in early November following a killing frost of -2 °C to a stubble height of 1.5

cm. Vegetation samples were dried at 60 °C. These samples were then ground with a Wiley mill (Thomas-Wiley Mill Co., Philadelphia, PA, USA) through a 1-mm screen and then reground with a cyclone mill. All harvested material was analyzed for biomass along with %N and %P in the tissue (Current et al. 2016). Samples were submitted to Brookside Laboratories in New Bremen, OH, or Agvise Laboratories in Benson, MN. Percent phosphorus was determined using inductively coupled plasma mass spectroscopy and percent nitrogen in the tissue was determined with dry combustion and a Perkin-Elmer 2400CHNS Analyzer. Biomasses and nutrient measurements were extrapolated to the entire wetland area on the years when only quadrats were harvested.

Load Calculations and FLUX

FLUX software was used to calculate the load of each nutrient flowing into and out of the wetland (Walker 1996). Inputs into FLUX were average daily flow rate (cfs), nutrient concentrations from grab samples (mg L^{-1}), and flow rates at the time of the grab sample (cfs). The flow weighted mean concentration was used for estimating the load each year in FLUX. In 2015 and 2016, these calculations were stratified into two seasons, February-July and August-December. These months were chosen due to the clear distinctions in nitrate concentrations between those periods and the dry period in July and August each year separating the two periods. The loading rates into the wetland (kg ha^{-1} yr^{-1}) were then calculated by dividing the mass flowing into the wetland each year by the wetland's watershed area.

Results

General Hydrology

In each of the first three years of the study, the wetland received average rainfall amounts for the region (66.7-79.6 cm for the full year), but it received more rainfall than an average year in 2016 (University of Minnesota 2017; Table 1 & Figure 8). Although 2015 had the lowest tile inflow of the three years, it had the most rainfall between the time the ground thawed in the spring and froze in the fall. The first two years had a few large rain events which lead to a large portion of the volume coming from large flow events. The third year had a greater total rainfall but fewer large storm events. Because of the large events in 2013 and 2014, the wetland flooded in June both years (Figure 8). Elm Creek flooded over its banks and some water flowed into the wetland through the outlet pipe. However, flood water did not exceed the height of the berms.

Evapotranspiration was a small fraction of the loss of water from the wetland (Table 2). In 2015, the third cell was never inundated and received water from the second cell for only a couple of hours. Therefore, evapotranspiration in the third cell played a negligible role in the 2015 water budget.

Table 1. Rainfall amounts contributing to the wetland water budget during the period from the ground thawing in the spring to freezing in the fall each of the four years of the study. Error ranges for volumes are in parentheses.

Year	**Rainfall (cm)**	**Rainfall Volume into Wetland (m^3)**
2013*	30.71	337.0 (333.6-340.4)
2014	46.53	510.7 (505.6-515.8)
2015	61.44	674.3 (667.5-681.0)
2016	91.80	1,007 (997.3-1,018)

* In 2013, the tile was not connected to the wetland until the end of May, so the rainfall amount in the table was from May 1, 2013, until freezing in the fall.

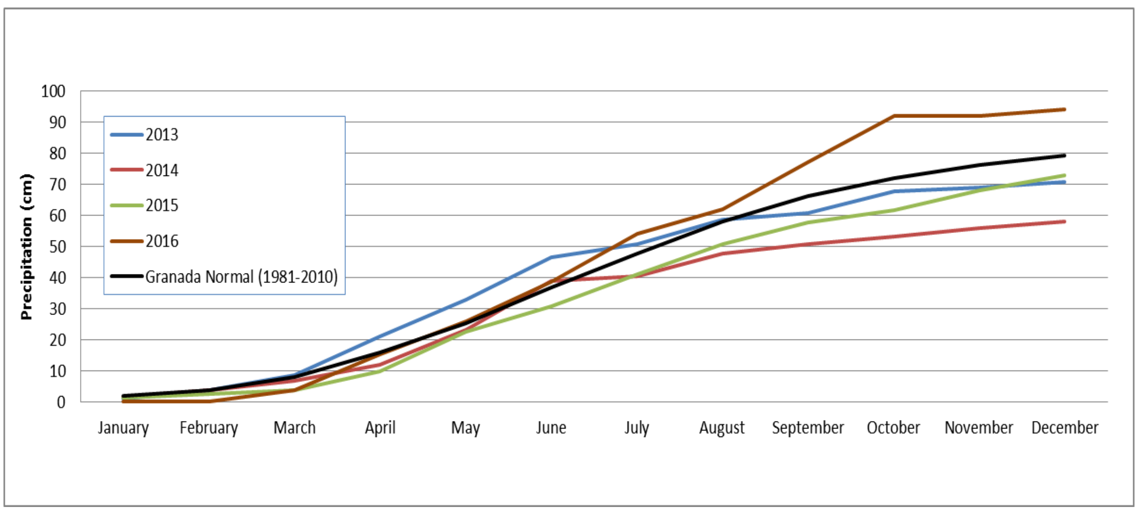

Figure 7. Cumulative monthly precipitation at the study site from 2013 to 2016 and the normal precipitation curve for Granada, MN (University of Minnesota 2017).

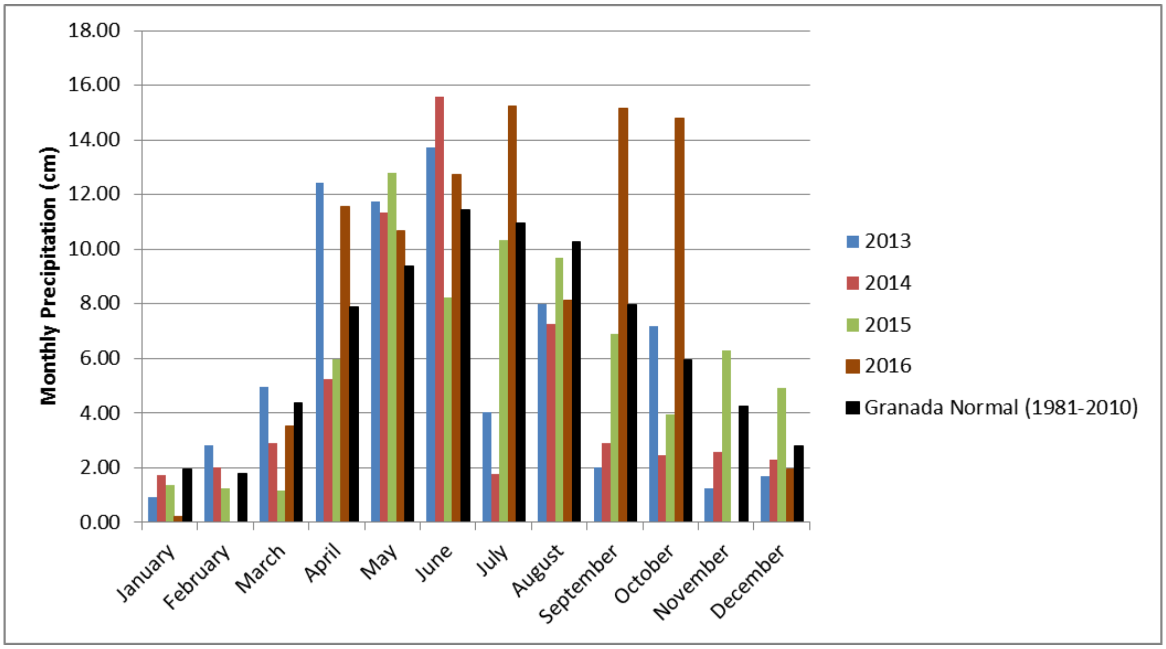

Figure 8. Monthly precipitation totals at the wetland site and the Granada Normal (1981-2010) (University of Minnesota 2017).

Table 2. Calculated potential evapotranspiration from two equations. Each mean and standard deviation (SD) was from the first day of flow into the wetland to the final day of flow that year.

Year	Hamon method (SD, mm day^{-1})	Hamon method (m^3)	Thornthwaite method (SD, mm day^{-1})	Thornthwaite method (m^3)
2013	2.4 (1.2)	475	3.0 (1.9)	610
2014	3.0 (1.2)	667	3.8 (2.0)	849
2015[a]	2.8 (1.1)	492	3.4 (1.9)	601
2016	3.0 (1.7)	928	3.1 (2.2)	951

[a]Evapotranspiration in 2015 is estimated for cells 1 and 2 only due to cell 3 only receiving 6 m^3 of water from tile flow that year.

Surface Hydrology

The greatest input of water into the wetland was tile drainage. The volume of this input varied in each of the four years, 2013 through 2016, that this wetland was studied (Table 3 & Figure 9). The fourth year, 2016, had the greatest number of days of inundation (Table 4).

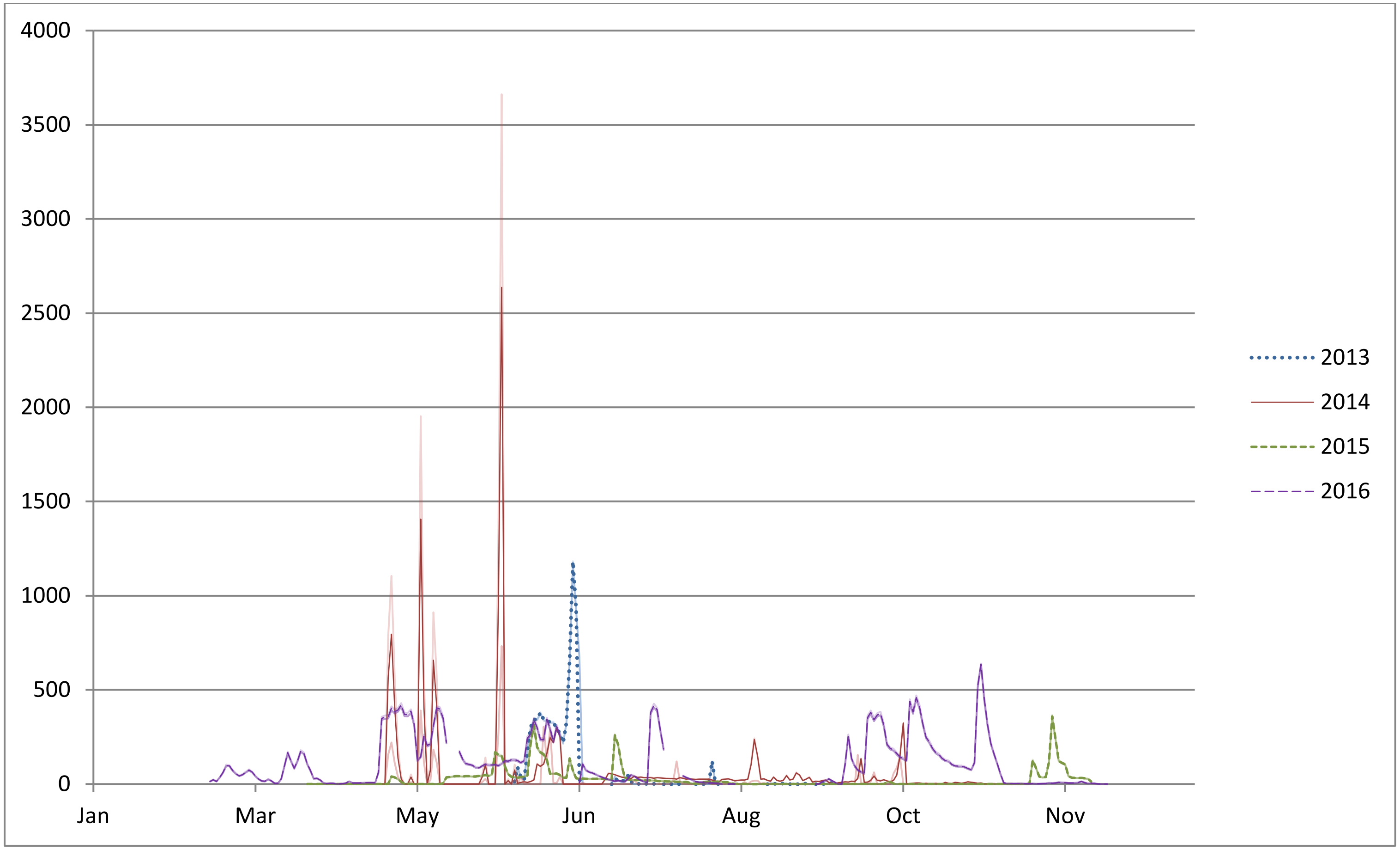

Figure 9. Hydrograph of the tile inflow entering the wetland during all four years of the study. Error limits are displayed as shaded lines above and below each year's data.

Table 3. Volume of water flowing into the wetland from tile drainage each year of the study. The flow period is listed by start and end dates each year. Error ranges for inflow volumes are in parentheses.

Year	Tile Drain Inflow Volume (m^3)	Starting Date of Flow	Approximate Last Date of Flow
2013*	7,240 (7,107-7,267)	June 6	September 11
2014	13,750 (12,210-17,260)	April 27	October 30
2015	6,307 (6,291-6,323)	April 28	December 1
2016	27,970 (27,880-28,030)	March 1	December 2

*Measurements began in late May of 2013. Spring flow events are not included in this estimate.

Table 4. Number of days of flow into the wetland and inundation observed in each wetland cell. This was defined as the number of days water was covering the entire cell and reached the control structure leading out of the cell.

Flow Statistic	**Year**				**Average**
	2013	**2014**	**2015**	**2016**	
Flow into wetland	54	182	98	231	141
Inundation Cell 1	28	88	17	133	67
Inundation Cell 2	19	43	7	95	41
Inundation Cell 3	13	31	0	91	34
Flow out of wetland	13	27	0	63	26

Infiltration

The largest output from wetland flowed out by infiltrating into the shallow groundwater. Approximately 76%, 46%, and 70% of the water infiltrated in 2013, 2014, and 2016. Approximately 93% of the water in the wetland infiltrated into the shallow groundwater in 2015 (Figure 10). No water that year flowed through the surface outlet.

Table 5. Summary of the inflow and outflow volumes in the wetland each year. The water volume ranges display high variability in some cases based on the range of the respective instruments and calculations' accuracies.

	2013		2014		2015		2016	
	Water Volume	**Water Volume Range**	**Water Volume**	**Water Volume Range**	**Water Volume**	**Water Volume Range**	**Water Volume**	**Water Volume Range**
INFLOWS (m^3)								
Tile Inflow	7,250	7,110-7,270	13,800	12,200 – 17,300	6,310	6,290-6,320	28,000	27,900-28,000
Rainfall	337	334-340	511	506 – 516	674	668-681	1,030	997-1,000
OUTFLOWS (m^3)								
Surface Outlet Drain	1,310	1,300-1,340	7,000	6,820-7,380	0.0	0.0	7,680	7,650-7,720
Evapotranspiration	475	475-569	667	667-671	492	482-492	928	809-928
Infiltration	5,800	5,530 – 5,830	6,470	4,660-10,300	6,490	6,470-6,520	20,400	20,200-20,600

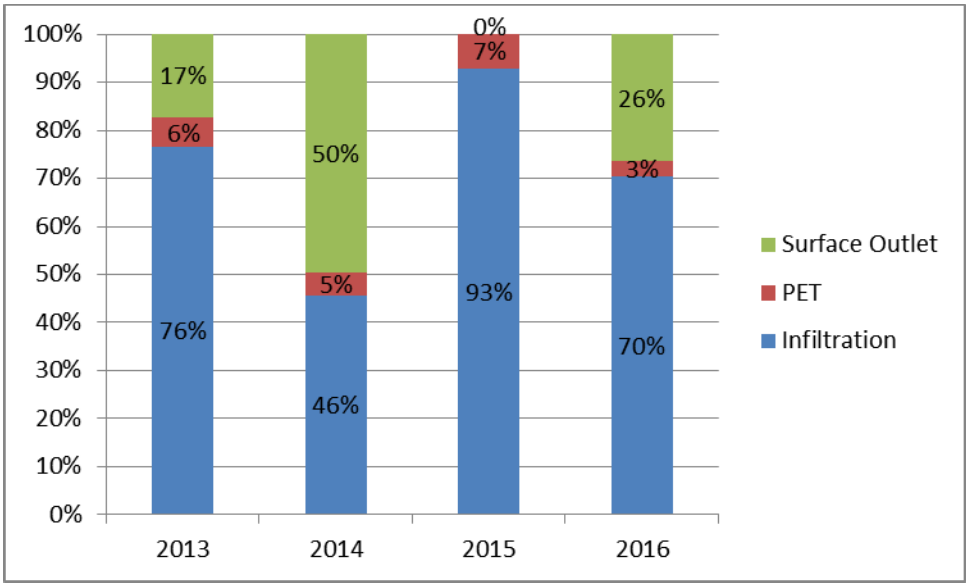

Figure 10. Outflow distribution of all water which entered the wetland each year. The total water volume includes precipitation.

The measured vertical Ksat in the two infiltrometers were 0.23 and 0.15 m day^{-1}. The average measured Ksat was therefore approximately 0.19 m day^{-1} (Table 6). The vertical Ksat estimated through the water balance approach in the spreadsheet model in 2013, before vegetation established and soil settled completely, was approximately 0.22 m day^{-1}. The average vertical Ksat estimated by balancing the water budget (Equation 1) from 2014 through 2016 was 0.17 m day^{-1}. The average horizontal Ksat in the shallow groundwater layer was 1.2 m day^{-1} from the slug tests in the piezometers. The average Darcy flux was 0.012 m day^{-1}.

Table 6. Average saturated hydraulic conductivity (Ksat) in the wetland.

	Saturated Hydraulic Conductivity (Ksat; m day^{-1})
Measured Vertical	0.19
Water Balance-Calculated Vertical	0.17
Horizontal	1.2

Shallow Groundwater Flow

The primary direction of flow was from the southwest toward the northeast (Figure 6). When the wetland was inundated, water would infiltrate through the topsoil into the shallow groundwater and then flow toward Elm Creek.

Results from the stable isotopes, δD and $\delta^{18}O$, were inconclusive. Isotopes from the tile drainage and shallow groundwater from the edge of the wetland and center of the wetland could not be distinguished. Results from the SC measurements were also inconclusive when attempting to distinguish the sources of water in each well. However, the SC values in the wells never exceeded 1,000 µs cm^{-1}, even when the surface of the wetland was dry. The SC measurements in the shallow groundwater of the wetland therefore never reached the baseline SC from the three deep groundwater SC measurements from the site in 2012 [mean = 1,250 µs cm^{-1}, standard deviation (SD) = 352].

Residence Time

The retention time when surface water was flowing through the entire wetland ranged from approximately 1 to 24 days (25th – 75th percentiles) with the median retention time being 1.9 to 10.3 days. Flow through the first cell ranged from approximately 4 hours to 7.2 days (25^{th} – 75^{th} percentiles; Appendix 1, Table 31). All these times depended on the flow rate and volume of water entering the wetland which fluctuated greatly. Due to most of the water infiltrating, the surface water residence time reflects only a portion of the residence time.

Average retention times in the shallow groundwater flow ranged from 36.6 to 200 days depending on the cell from which the water infiltrated and the year (Table 7). Most of the water entering the wetland infiltrated into the shallow groundwater from within cell 1 before it reached the first Agri Drain control structure. From that cell, residence time in the shallow groundwater flowing to the creek was on average 182 days.

Table 7. Average shallow groundwater residence times after infiltrating from the wetland surface until reaching the creek.

Year	**Cell**	**Mean Residence Time (SD, days)**
2013	1	200 (29.8)
	2	107 (13.6)
	3	42.1 (4.3)
2014	1	175 (42.7)
	2	101 (21.6)
	3	40.1 (7.7)
2015	1	198 (21.8)
	2	110 (3.1)
	3	NA
2016	1	156 (54.4)
	2	88.3 (27.7)
	3	36.6 (9.6)

Nutrient Loads

The greatest load of nutrients entering the wetland was observed in 2016 (Table 8). Although 2015 had more rainfall than the two previous years, it had the smallest nutrient load. The final year had the greatest rainfall, greatest volume of water entering the wetland, and the greatest nutrient loads (Table 8). Although 2016 had the lowest mean concentration of nutrients, it still had the greatest load.

Due to the lack of water discharging from the outlet in 2015 (Table 9), that year had no nutrients discharging into the creek through the surface outlet. Although 2016 had the greatest load entering the wetland of the four years, 2014 had a greater load of nitrate discharging into the creek. In 2016, however, there was more phosphorus discharging into the creek than any of the previous years.

Table 8. Flux estimates of inflow volumes and nutrient loads for each year based on flow weighted concentrations.

	Total Days of Monitoring	Total Volume of Water (m^3)	Nitrate/Nitrite-N Load (c.v., kg)	Total Phosphorus Load (c.v., kg)	Soluble Orthophosphorus Load (c.v., kg)
2013[1]	94	8,000	185 (0.029)	0.419 (0.13)	0.320 (0.16)
2014	209	13,800	213 (0.092)	2.09 (0.15)	1.65 (0.12)
2015	242	6,320	87.9 (0.035)	0.251 (0.13)	0.220 (0.066)
2016	264	28,000	252 (0.039)	2.43 (0.50)	1.08 (0.12)

[1]2013 inflow did not begin until June 6th.

Table 9. Flux estimates of surface outflow volumes and nutrient loads flowing out of the wetland each year based on flow weighted concentrations.

	Total Days of Monitoring	Total Volume of Water (m^3)	Nitrate/Nitrite-N Load (c.v., kg)	Total Phosphorus Load (c.v., kg)	Soluble Orthophosphorus Load (c.v., kg)
2013	89	1,860	41.4 (0.14)	0.201 (0.44)	0.092 (0.71)
2014	203	7,080	91.3 (0.056)	0.934 (0.22)	0.556 (0.23)
2015	242	0	0	0	0
2016	222	8,960	59.1 (<0.01)	2.10 (0.49)	0.851*

*There were not enough grab samples of soluble orthophosphorus in 2016 for Flux to calculate the load. This value is the average of the two grab samples multiplied by the total volume of water leaving the outlet.

Table 10**.** Flow weighted mean nitrate/nitrite-N concentrations flowing from the tile drain into the wetland each year. Concentrations were divided into two seasons due to the significant differences between the concentrations in the two seasons each year.

	Nitrate/Nitrite-N (FLUX c.v., mg L^{-1})			
	2013	**2014**	**2015**	**2016**
April 14 - June 30	23.2 (0.03)	15.4 (0.09)	15.1 (0.05)	11.0 (0.06)
July 1 - November 30	NA[a]	26.0 (NA[a])	11.8 (0.05)	7.3 (0.06)

[a]No data available during this season.

Table 11**.** Flow weighted mean soluble orthophosphorus concentrations flowing from the tile drain into the wetland each year. Concentrations were divided into two seasons due to the significant differences between the concentrations in the two seasons each year.

	Orthophosphorus (FLUX c.v., mg L^{-1})			
	2013	**2014**	**2015**	**2016**
April 14 - June 30	0.040 (0.16)	0.120 (0.30)	0.034 (0.069)	0.004 (0.12)
July 1 - November 30	NA[a]	NA[a]	0.033 (0.069)	0.039 (0.12)

[a]No data available during this season.

Table 12. Flow weighted mean total phosphorus concentrations flowing from the tile drain into the wetland each year. Concentrations were divided into two seasons due to the significant differences between the concentrations in the two seasons each year.

	Total Phosphorus (FLUX c.v.; mg L^{-1})			
	2013	**2014**	**2015**	**2016**
April 14 - June 30	0.052 (0.13)	0.152 (0.28)	0.048 (0.14)	0.032 (0.75)
July 1 - November 30	NA[a]	NA[a]	0.038 (0.14)	0.193 (0.75)

[a]No data available during this season.

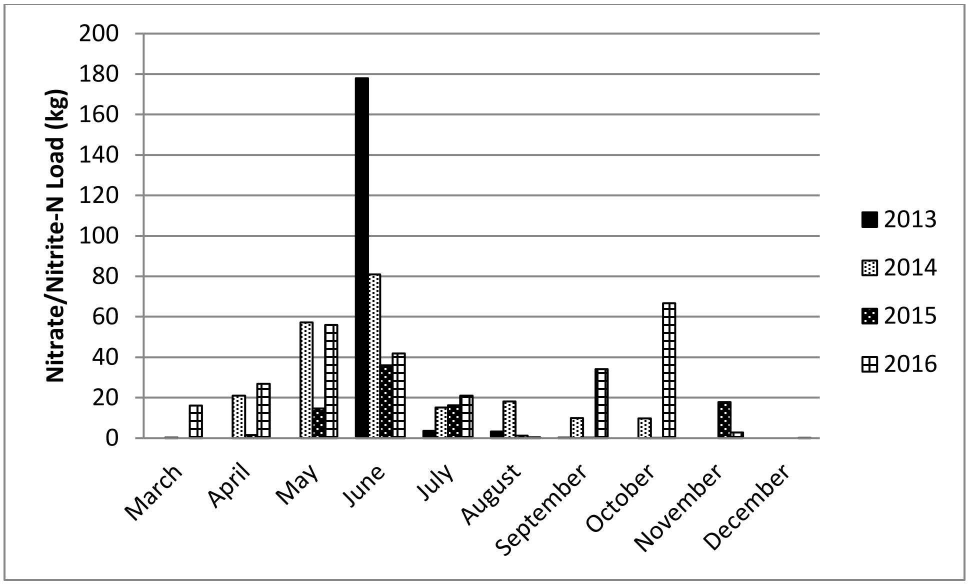

Figure 11. Summary of nitrate/nitrite-N loads into the wetland each month in each of the four years.

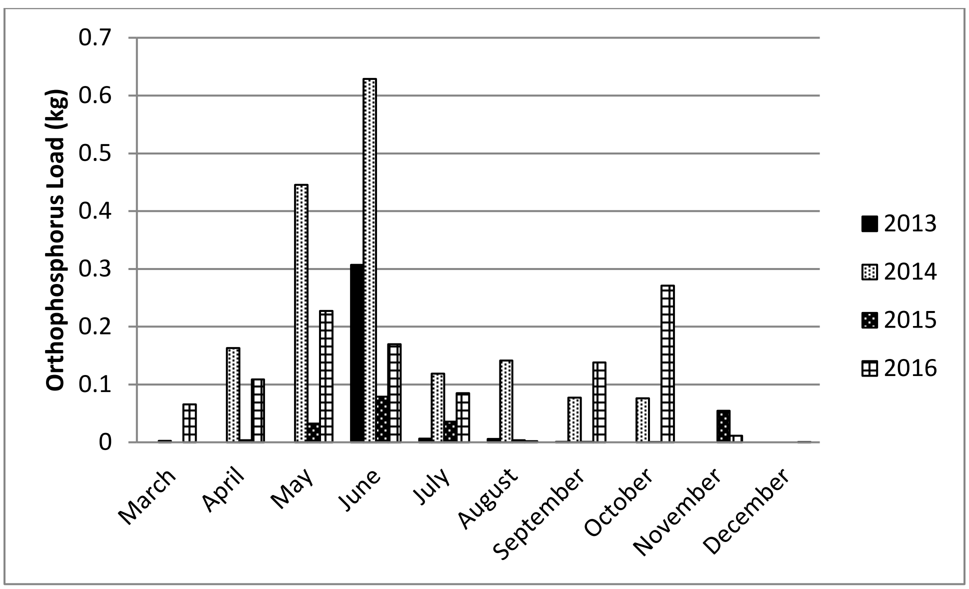

Figure 12. Summary of orthophosphorus loads into the wetland each month in each of the four years.

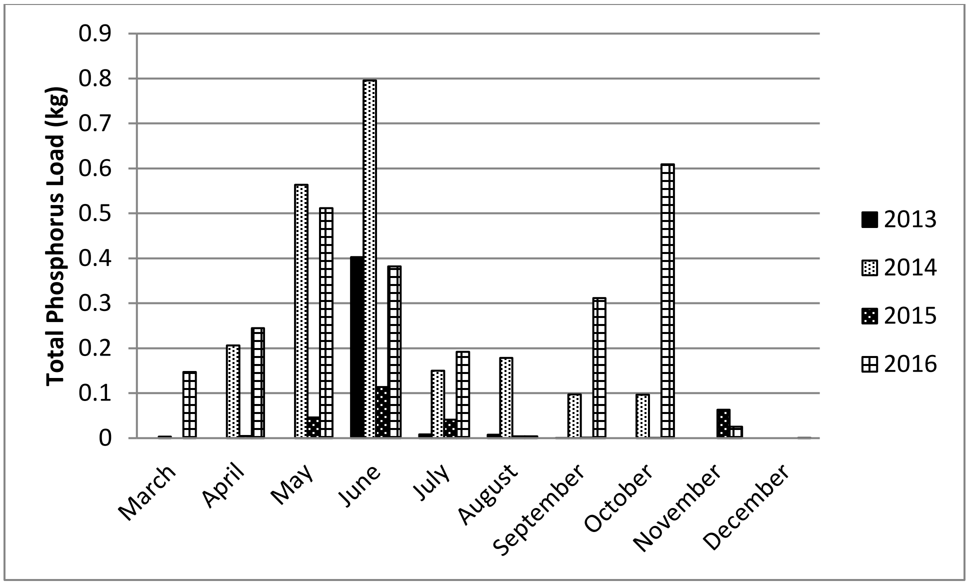

Figure 13. Summary of total phosphorus loads into the wetland each month in each of the four years.

June was the month with the greatest load of nutrients each year. This month was followed by May, but other months were inconsistent in relative loads (Figure 11-Figure 13). The average annual nitrate-N concentration flowing into the wetland decreased each year from 23.2 mg L^{-1} in 2013 to 9.5 mg L^{-1} in 2016 (Table 10). Orthophosphorus and total phosphorus concentrations varied more than nitrate-N concentrations each year (Table 11). Average seasonal total phosphorus concentrations were as high as 0.193 mg L^{-1} in the second half of 2016 to as low as 0.032 mg L^{-1} in the first half of 2016 (Table 12). Orthophosphorus was 76-79% of the total phosphorus concentration in 2013-2015 and 44% of the total phosphorus in 2016. The fourth year, 2016, had the greatest overall load of nutrients, but that load was more evenly distributed throughout the growing season than in other years. Although the load was more evenly distributed, 2016 was still the year of lowest reduction percentage. Furthermore, the residence time was positively correlated with the percent removal of nitrate. As residence time increased, the percent removal increased. Thus, 2015 had the greatest percent removal, lowest hydraulic loading rate, and greatest residence time. In the 10.1-ha watershed, the average nitrate/nitrite-N load leaving the tile drains was 18.2 kg ha^{-1} (Table 13). Total nitrate removal in the wetland averaged 67% (Table 13), and the concentration reductions of nitrate from the surface inlet to surface outlet ranged from 3.9 to 34.8% each year (Table 14).

Table 13. Loading rates of nitrate/nitrite-N entering the wetland each year and calculated reduction in the wetland.

Year	Annual Nitrate/Nitrite-N Load (kg ha^{-1} yr^{-1})	Hydraulic Loading Rate (m yr^{-1})	Surface and Shallow Groundwater Nitrate Load Reduction
2013	18.3	6.6	70%
2014	21.0	12.5	48%
2015	8.7	5.7	100%
2016	24.9	25.5	70%
Four-Year Total	18.2	12.6	67%

Table 14. Surface reduction of nitrate/nitrite-N concentration.

Year	Surface Nitrate Reduction	Percent of Water Volume Leaving Surface Outlet
2013	3.9%	17.3%
2014	34.8%	49.1%
2015	NA	0%
2016	26.7%	26.5%
All Years	10.2%	27.7%

Biomass harvested from each quadrat from 2013 to 2015 ranged from 0.2 to 9.8 Mg ha^{-1} dry weight and increased each year. Cell 3 had the greatest biomass in 2013 and 2014, but Cell 1 had the greatest biomass by 2015. The mass of nitrogen measured in each quadrat's harvested vegetation ranged from 0.8 to 80.5 kg N ha^{-1}, and the mass of phosphorus ranged from 0.2 to 11.5 kg P ha^{-1} (Table 15).

The mass of nitrogen available for harvest with the vegetation was less than 1% of the mass of nitrogen entering the wetland each year except in 2015, when it was 3% of the mass (Table 16). Therefore, the harvest of vegetation had a minimal impact on nitrogen removal in this wetland. However, the portion of phosphorus available for harvest was much greater. In the first three years, 20%, 23%, and 189% of the inlet load of phosphorus could have been removed (Table 20). In the third year, more phosphorus could have been harvested than what entered the wetland. However, it was not actually harvested that fall, so the phosphorus remained in the system. If the vegetation had the same growth in 2016 as the previous two years, harvesting it would have removed 29% of the phosphorus input from tile drainage.

Table 15. Calculated uptake of nitrogen and phosphorus in vegetation averaged by wetland cell.

Year	**Cell**	**N (kg ha^{-1})**	**SD**		**P (kg ha^{-1})**	**SD**
2013	1	1.5	0.61		0.44	0.18
2013	2	2.2	0.93		0.67	0.29
2013	3	7.6	3.9		1.3	0.65
2014	1	15	2.5		3.6	0.44
2014	2	13	4.0		3.6	1.0
2014	3	26	7.3		6.3	2.4
2015	1	40	21		5.1	1.8
2015	2	16	7.3		3.7	1.6
2015	3	18	4.7		4.4	1.4

Table 16. Calculated masses and distributions of nitrate/nitrite-N in the wetland each year.

	2013		2014		2015		2016	
	Mass of Nitrate/ Nitrite-N (kg)		**Mass of Nitrate/ Nitrite-N (kg)**		**Mass of Nitrate/ Nitrite-N (kg)**		**Mass of Nitrate/ Nitrite-N (kg)**	
INFLOW								
Tile Inflow[a]	185	c.v.=0.029	213	c.v.=0.092	87.9	c.v.=0.035	252	c.v.=0.039
OUTFLOW								
Surface Outlet Drain[a]	41.4	c.v.=0.14	91.3	c.v.=0.056	0	NA	59.1	c.v.<0.01
Shallow Groundwater Flow to Creek[b]	15.1	11.8 – 21.9	18.4	15.5 – 23.4	0.0	NA	17.2	13.7 – 25.8
REMOVAL								
Surface and Subsurface Removal	128	NA	101	NA	85.3	NA	176	NA
Harvest from Vegetation	0.405	c.v.=0.38	1.92	c.v.=0.089	2.63	c.v.=0.10	NA[c]	NA

[a]The c.v. was calculated in FLUX for the Tile Inflow and Surface Outlet Drain.

[b]Shallow groundwater error ranges are calculations using the lowest and highest K reaction coefficients measured in the shallow groundwater (0.0194 and 0.0284 day^{-1}).

[c]Nitrogen assimilation in the vegetation was not measured in 2016.

Due to the large volumes of water infiltrating into the shallow groundwater, a large portion of the soluble nutrients also infiltrated into the shallow groundwater. Up to 77%, 56%, 97%, and 77% of the nitrate loads likely infiltrated into the shallow groundwater each of the four years. Reductions of the infiltrated mass then ranged from 85 to 100%. All the nitrate that infiltrated into the shallow groundwater was removed in 2015.

Table 17. Flow-weighted mean concentrations of total phosphorus from the surface inlet and surface outlet each year.

Year	**Surface Inlet (c.v., mg L^{-1})**	**Surface Outlet (c.v., mg L^{-1})**
2013	0.052 (0.13)	0.11 (0.44)
2014	0.15 (0.28)	0.13 (0.22)
2015	0.043 (0.14)	NA
2016	0.087 (0.64)	0.23 (0.49)

Table 18. Load of total phosphorus entering the wetland each year.

Year	**Annual Total Phosphorus Load (kg ha^{-1} yr^{-1})**	**Hydraulic Loading Rate (m $year^{-1}$)**
2013	0.041	6.6
2014	0.21	13
2015	0.025	5.7
2016	0.24	26
Four-Year Total	0.13	13

There was an increase in total phosphorus concentrations from the inlet to the outlet on the surface of the wetland (Table 17). Total phosphorus concentrations in the wells were also consistently greater than concentrations in the tile drainage flowing into the wetland. Although the concentration across the surface increased, the load of total phosphorus discharging through the surface outlet was less than that entering the surface due to much of the water infiltrating into the shallow groundwater (Table 19 & Table 20).

The mass of phosphorus in the top 0.5 meters of soil also decreased an average of 0.98 kg yr^{-1} (Table 19). The phosphorus released from the wetland was approximately 1.6 times more than what flowed into it over the first four years of the wetland. Most of the phosphorus lost was released from the soil. If the soil phosphorus were not released, the wetland would have removed 37.8% of the phosphorus in the first three years – all removed through harvested vegetation. By 2018, after the hydrologic monitoring period analysis was complete, the soil phosphorus was less than 2.0 mg kg^{-1}, so the phosphorus loss would be greatly diminished after 2018.

Table 19. Soil phosphorus measurements from the wetland and estimates of soil phosphorus in the top 0.5 meters at the time of the sample.

Year	Mean Soil Phosphorus Concentration (mg kg^{-1})	Total Wetland Soil Phosphorus (kg)
2012	10.6 (5.1)	6.9
2014	6.0 (NA*)	3.9
2018	1.6 (0.8)	1.0

*All samples were mixed for one analysis.

Table 20. Calculated masses and distributions of total phosphorus in the wetland each year.

	2013		2014		2015		2016	
	Mass of phosphorus (kg yr^{-1})	**c.v.**	**Mass of phosphorus (kg yr^{-1})**	**c.v.**	**Mass of phosphorus (kg yr^{-1})**	**c.v.**	**Mass of phosphorus (kg yr^{-1})**	**c.v.**
INFLOW								
Tile Inflow[a]	0.419	0.13	2.09	0.15	0.251	0.13	2.43	0.50
OUTFLOW								
Surface Outlet Drain[a]	0.201	0.44	0.934	0.22	0.0	0.0	2.10	0.49
Shallow GW Flow to Creek[b]	1.11		1.65		0.752		1.31	
REMOVAL								
Harvest from Vegetation	0.0854	0.32	0.484	0.26	0.474[c]	0.22	NA[d]	NA

[a]The c.v. was calculated in the FLUX software for the tile inflow and surface outlet drain.

[b]The Shallow GW Flow to Creek is the difference between the inflow and soil P lost and the measured outflow and removal (tile inflow + annual soil P lost (0.98 kg yr^{-1}) - [surface outlet drain + harvest from vegetation] = Shallow GW flow to Creek).

[c]Not all the vegetation in 2015 was harvested. Therefore, this mass remained in the wetland and contributed to the mass lost in 2016.

[d]Vegetation was not harvested in 2016.

Discussion

The objective of this study was to evaluate the effectiveness of an edge-of-field nutrient treatment wetland that is smaller than most nutrient treatment wetlands in the Midwest and determine the effect of infiltration into shallow groundwater and harvesting of vegetation on its effectiveness. In order for a constructed wetland to meet funding considerations for the Iowa CREP program, the watershed must be at least 200 ha (Hyberg et al. 2015; IDA 2009). The constructed wetland in this study treats tile drainage from 10.1 ha of corn-soybean rotation. Although the surface of the wetland was sealed with clay from the construction site, most of the water that entered this wetland flowed out through infiltration. When the wetland was constructed, the vertical Ksat was supposed to be closer to 8.64×10^{-5} m day^{-1} on the surface based on recommendations in the National Engineering Handbook (USDA NRCS 2009), but it ended up being between 0.17 and 0.19 m day^{-1}. After measuring reductions of nitrate in the shallow groundwater, infiltration in the wetland increased residence time and denitrification.

Although this wetland was designed differently than others, it performed comparably to other nutrient removal wetlands. Christianson et al. (2013) summarized the reductions from eight other tile drainage treatment wetlands which removed a mean of 42.8% of nitrate with a quartile range of 30.9-55.0%. Nutrient reduction strategies in the Upper Midwest assume treatment wetlands will reduce 50% of nitrate (IDALS 2016b; IEPA 2014; MPCA 2014). This wetland removed 67% of the nitrate entering it with a range of 48-100% in the four years of the study. Wetlands in the Iowa CREP program are expected to remove 40-90% of the nitrate entering them (Crumpton and Stenback 2016; Hyberg et al. 2015). Therefore, the wetland in this study is comparable to or slightly better than the average treatment wetland in the Midwest.

While the most biologically active area is in the top 10 cm of soil (Brauer et al. 2015), denitrification still occurs below that surface (Jaynes and Isenhart 2014). The potential reductions in shallow groundwater are oftentimes overlooked but have increased the effectiveness in some treatment wetlands (Brauer et al. 2015; Larson et al. 2000). Over the course of this study, 56-97% of the nitrate infiltrated into the shallow groundwater. Of the nitrate that infiltrated, 85-100% was then removed. Whereas the reduction of the nitrate concentration on the surface was approximately 28%. The shallow groundwater reduction, therefore, became a vital part of the wetland's effectiveness.

This wetland was not designed to remove phosphorus. Wetlands constructed for phosphorus removal are oftentimes designed for sedimentation (Kadlec and Wallace 2008). In this wetland, tile drainage had minimal concentrations of sediment and attached phosphorus. Therefore, the first cell did not serve as a settling pond in this wetland. However, recent observations of phosphorus sources in places like the Lake Erie basin have driven the need for more BMPs that can reduce dissolved phosphorus in subsurface drainage (King et al. 2015; Smith et al. 2015). Total phosphorus loads discharging from the tile drain in this study (0.025-0.24 kg ha^{-1} yr^{-1}) were lower than the typical annual load of 0.4 to 1.6 kg ha^{-1} yr^{-1} in the Lake Erie basin (King et al. 2015). Removal of phosphorus in treatment wetlands may differ at locations where the phosphorus load is greater. However, the impact of vegetation harvest in this type of wetland and plant community is applicable for other locations.

Phosphorus cannot be removed from the soil or water as a gas similarly to nitrogen. In the wetland in this study, the primary means of phosphorus removal was through vegetation harvest. In the three years when nutrient composition in the vegetation was measured, 20-189% of the input phosphorus could have been removed. In 2015, more phosphorus could have been removed through vegetation harvest than what entered the wetland. In the years with high loading rates, vegetation harvest still would have removed at least 20% of the phosphorus. However, in the four years of this study, approximately 1.6 times more phosphorus was released to the creek than what entered the wetland. The concentrations of phosphorus also increased on the surface and in the shallow groundwater as they flowed toward Elm Creek. This likely was due to the high concentration of phosphorus in the soil reaching equilibrium with the low concentration of phosphorus in the water and phosphorus being released from iron binding sites once the soil became anaerobic (Hoffmann et al. 2009; Kronvang, Hoffmann, and Dröge 2009). The soil phosphorus decreased over the course of the study until it reached a concentration in 2018 at which it is unlikely to release significant masses into the water column in future years. If phosphorus is no longer released from the soil, harvesting the vegetation would reduce the load of phosphorus moving through the wetland each year. The kg ha^{-1} of phosphorus available for harvest is lower in this wetland than in other studies (Grosshans et al. 2014; Skłodowski et al. 2014), but these other studies measured phosphorus from highly productive species such as *Typha sp.* or *Salix sp.* rather than diverse wet prairie communities.

Conclusion

This wetland removed 67% of nitrate-N discharging from tile drainage, but it released phosphorus during its first four years after construction. However, it is likely to be more effective at removing phosphorus through vegetation harvest because almost all the soil phosphorus was released. The hypothesis that infiltration of nitrate into the shallow groundwater would increase the effectiveness of this wetland was confirmed because most of the water that infiltrated into the shallow groundwater was removed before it reached Elm Creek.

There are some areas where design and management could be improved for those who construct a similar design in the future. The wetland could be more effective if the outlet boards are raised high enough to hold more water and allow more infiltration. This wetland was also a unique case where university scientists managed the flow and vegetation each year. Therefore, other management strategies will need to be considered, whether the farmer or local county staff manage these components. Finally, those who constructed this wetland attempted to seal it with a clay liner, but it may be better to plan on both surface and shallow groundwater flow. In locations where groundwater contamination is a concern, it may be important to seal the bottom, but then it may not perform as well as this wetland. Furthermore, the shallow groundwater reductions in this wetland indicate there may not be a concern for groundwater contamination after all.

Future studies could focus more on the potential of harvesting vegetation for more phosphorus removal. This wetland was designed to observe the effectiveness of a wet-prairie mix wetland as a multi-functional system. While vegetation harvest was being studied for nitrogen and phosphorus removal from the wetland, a parallel study was measuring the harvest's potential for biofuels. The hypothesis was confirmed that harvesting the vegetation from this wetland played a major role in its removal of phosphorus. Other studies could therefore focus on what adjustments could be made to the seed mix or harvest regime to improve phosphorus removal. Other plant communities could also be studied in this system. Because the wetland is not inundated as often as marshes where highly productive species such as *Typha sp.* grow, species that are adapted to fluctuating water levels would be best.

Chapter 2: Nitrogen reductions and microbial populations in *Phalaris arundinacea* and wet prairie mix wetland mesocosms

Abstract

Reed canary grass (*Phalaris arundinacea* L.) is an invasive, cool-season grass commonly dominating wetlands with high nutrient loads. While it is highly productive, it is unknown whether it increases or decreases nitrogen removal from wetlands it invades. Nitrate removal was compared among wet prairie mix, switchgrass-dominated, and reed canary grass-dominated communities in laboratory mesocosms. The wet prairie mix communities on average removed more nitrate in each test than the switchgrass and reed canary grass mesocosms ($p = 0.01$ & 0.08). While the wet prairie mix removed the most nitrate from the surface water ($p < 0.01$), the reed canary grass removed more from the subsurface ($p < 0.01$). The dissolved oxygen was negatively correlated with nitrate removal in the mesocosms ($p < 0.001$), but reed canary grass had the greatest subsurface removal despite having a high dissolved oxygen concentration. This may be due to the reed canary grass assimilating nitrogen and oxygenating the rhizosphere after the other communities had already senesced. This observation was supported when using qPCR analysis to quantify the 16S rRNA genes to estimate the total bacteria population and *nosZ*1 and *nosZ*2 denitrifying enzyme primers to estimate the denitrifying bacteria population. The ratios of denitrifying to total bacteria were significantly higher in the wet prairie mix root zone than the other communities' root zones ($p < 0.05$). This provided further evidence that the wet prairie mix community provided a better environment for

denitrification and, thus, greater nitrate removal. The results, therefore, suggest that reed canary grass invasion could reduce the effectiveness of wetlands at removing nitrogen.

Introduction

Hydrology is arguably the most important feature to consider when designing a wetland to remove nitrate from tile drainage (Kadlec and Wallace 2008). While hydrology is foundational, the plants and microbes in the wetland are removing the nitrate. To have a successfully designed edge-of-field treatment wetland, the living organisms cannot be overlooked. Many wetlands are being constructed primarily as agricultural best management practices (BMPs) for nutrient removal rather than for ecological restoration. In those wetlands, plant community management may not be a high priority for landowners or others managing the wetland. Therefore, it is important to know whether limited management will impact the effectiveness of these BMPs.

When constructing treatment wetlands, native vegetation is desired but invasive species such as *Phalaris arundinacea* L. (reed canary grass) often dominate over time if not managed (Eggers and Reed 2001; Stiles, Bemis, and Zedler 2008). The Natural Resources Conservation Service’s Constructed Wetland Practice Standard (CODE 656) does not allow invasive species in seed mixes and requires the removal of them after construction. However, the reason for this recommendation is usually to improve biodiversity (Cruse et al. 2012; Lenhart et al. 2017; Mcdonald et al. 2016; Wenzel and Sullivan 2012). Less is known about how reed canary grass impacts the effectiveness of treatment wetlands and other BMPs used to treat nitrate from tile drainage. Furthermore, little is known about whether invasive species like reed canary grass impact the denitrifying bacteria populations that are accomplishing most of the nitrate removal in treatment wetlands.

Reed canary grass is reported by the U.S. Army Corps of Engineers as one of the most destructive invasive species in the Upper Mississippi River system as it overwhelms native communities and reduces biodiversity (Guyon et al. 2012). As reed canary grass invades an area, it often creates a dense monoculture (Morrison and Molofsky 1998, Green and Galatowitsch 2001 & 2002, Maurer and Zedler 2002, Kercher and Zedler 2004). Although reed canary grass oftentimes leads to a monoculture or low-diversity community, it is usually a very productive plant, similar to other invasive species, which could in theory lead to better nutrient assimilation or available carbon for microbial communities involved in denitrification (Mitsch, Zhang, et al. 2005).

Exotic cool-season grasses with longer growing seasons, such as reed canary grass, will continue to be a problem for the native plant communities in any constructed wetland or ditch developed to treat nutrients from agricultural drainage (Crumpton et al. 2012). Even following establishment, these systems will likely need to be managed to prevent reed canary grass dominance due to high concentrations of nitrate tending to select for reed canary grass and the difficulty of removing reed canary grass once it establishes (Barrett 1989; Galatowitsch, Anderson, and Ascher 1999; Green and Galatowitsch 2001, 2002; Iannone and Galatowitsch 2008; Mack 1985; Morrison and Molofsky 1998; Perry, Galatowitsch, and Rosen 2002; Stiles et al. 2008; Teale 1982). If reed canary grass is invading and potentially converting many BMPs to monocultures over time, some questions are raised. First, how does reed canary grass compare to a native plant community in treatment wetlands? Second, what is the effect of each plant community's roots on the denitrifying environment in the soil?

Some previous studies have attempted to address these questions. David et al. (1997) looked at the effect of carbon availability and temperature on nitrate removal in bare soil mesocosms and reed canary grass mesocosms. They concluded that denitrification was likely the dominant mechanism for nitrate removal while temperatures enhanced the removal. However, they also concluded that the vegetation, reed canary grass, did not play a significant role because the bare soil samples had similar nitrate removal rates to soil containing reed canary grass. In another study, Herr-Turoff and Zedler (2005) compared the nutrient uptake of reed canary grass-invaded wetland plant communities to that of native wet prairie mixes due to the presumptions that reed canary grass retains a high level of nitrogen from wetland soil and water. However, they concluded that reed canary grass invasion did not increase nitrogen accumulation in the vegetation, had little effect on soil nitrogen, and did not remove more nitrate from water flowing through the wetland. Kreiling et al. (2015) measured an increase in nitrate availability in soils with reed canary grass compared to forested soils. That increase could mean reed canary grass has the potential to increase nitrate availability in wetlands instead of removing it. However, the inconsistencies in the results from studies measuring the impact of reed canary grass on nitrate reductions in wetlands demand more knowledge on the topic.

Studies addressing both the uptake of nitrogen by reed canary grass alongside the plant's interaction with denitrifying bacteria are limited. Studies have looked at the plant's nitrogen uptake and partial role in the nutrient cycle, but there is little research on the relationship between plant uptake and the denitrifying bacteria populations. Hoagland et al. (2001) found that approximately 90% of nitrogen removal is done by denitrification. As mentioned above, David et al. (1997), Xue et al. (1999), and Kreiling et al. (2011) also concluded that denitrification plays a larger role in nitrate removal than plant uptake. The rate of denitrification is thought to be related to denitrifying bacteria abundance (Baxter et al. 2012; O'Connor et al. 2006). Thus, if denitrifying bacteria abundance is lower in reed canary grass's rhizosphere, denitrification rates may also be lower. A higher C/N ratio in organic matter typically means it is lower quality tissue for decomposition and a slower decomposition rate. Therefore, if the C/N ratio in any plant community's tissue is higher, it could also lead to a lower denitrification rate in that community's rhizosphere (Bastviken et al. 2005; Capps et al. 2014; Cross et al. 2005; Enriquez, Duarte, and Sand-Jensen 1993; Martina and von Ende 2012).

There have been various methods used for estimating the denitrification rate in soils (Balderston, Sherr, and Payne 1976; Nason and Myrold 1991; Yoshinari, Hynes, and Knowles 1977). One method for estimating the denitrification potential of a soil is to quantify the denitrifiers present. This method involves extracting either DNA or RNA from the soil. These extractions can then be purified and amplified with the Polymerase Chain Reaction (PCR). Quantifying the 16S rRNA gene in soil samples can estimate how many total bacteria are present in the soil, but other primers targeting *nirS, nirK,* and *nosZ* can be used to estimate the population of denitrifiers. The potential amount of denitrification could then be based on the population of denitrifiers in the soil rather than a direct measurement of the conversion of NO_3^- to N_2O or N_2.

The *nosZ* gene is a key gene in denitrification for nitrous oxide reductase and is the most commonly used marker to quantify the denitrifying bacteria in soil samples (Baxter et al. 2012; Rich et al. 2003; Rösch, Mergel, and Bothe 2002). Each step in the denitrification process converting NO_3^- or NO_2^- to N_2O or N_2 gas has a specific enzyme or two enzymes associated with that step and a gene encoding the enzyme, as shown in the following pathway (Philippot, Hallin, and Schloter 2007; Rich and Myrold 2004):

Equation 14:

$$NO^-_3 \rightarrow^{\text{narG or napA}} NO^-_2 \rightarrow^{\text{nirS or nirK}} NO \rightarrow^{\text{qnorB or norB}} N_2O \rightarrow^{\textit{nosZ}} N_2$$

One objective of this portion of the study is to determine the role of different wetland plant types on nitrate removal in an edge-of-field agricultural treatment wetland. More specifically, it is to assess the impact of reed canary grass invasion on the effectiveness of nitrate reduction through a mesocosm and microbial DNA study. The hypothesis is that the reduction of nitrate will be significantly different between mesocosms containing native vegetation and those containing reed canary grass. The second objective is to determine if denitrifying bacterial populations differ among the root zones of each plant type. The final objective is to analyze various conditions that can potentially influence denitrification in edge-of-field tile drainage treatment wetlands including oxygen, pH, and carbon availability and how they are related to the plant types.

Materials and Methods

Experimental Design

The purpose of this study was to determine how the single factor, plant community type, within the Granada wetland impacted the effectiveness of the wetland at removing nitrate. Nine 380-liter mesocosm tanks were arranged in a complete randomized design based on a single factor with three levels. The dependent variables in this experiment were the change in nitrate concentration within the water of each mesocosm, the nitrogen uptake in plant tissue, and the denitrifying bacteria populations in the soil. Other variables were measured to confirm that there were no confounding variables in the experiment.

Mesocosm Study Setup

The Granada wetland is a small, edge-of-field treatment wetland located in Martin County 6.9 km north of Granada, MN (43.756562 N, 94.343852 W). The wetland was designed in 2012 and constructed in the winter and spring of 2013 (Karlheim 2012; Lenhart et al. 2016). Approximately 10.1 ha of subsurface tile drainage was rerouted to flow into the wetland.

The mesocosms were dispersed in the workshop of the Biosystems and Agricultural Engineering Building on the University of Minnesota St. Paul campus (1390

Eckles Avenue, St. Paul, MN 55108). This space had lighting on a timer, a large window to allow sunlight for most of the day, and easy access to watering. Most of the lighting came from the large windows, but the windows were complemented with full-spectrum bulbs over each tank. These bulbs were set to be on for 16 hours each day throughout the establishment period. Water came from a tap in the workshop. Because it was city tap water, it had substantial levels of chloramine (~2.5 mg L^{-1}; Gomez-Smith, LaPara, and Hozalski 2015). Therefore, before any water was used in the mesocosms, it was filtered through an activated carbon and zeolite filter to remove chlorine. Temperature was controlled with a thermostat, and the average temperature was 22.1 °C.

The 380-liter mesocosms were black, high-density structural resin stock tanks measuring 135 x 79 x 64 cm on the outside. The bottom 28 cm of each mesocosm were filled with glacial till sand from New Richmond, WI (TCC Materials®; Figure 14). A 1.3-cm hole was drilled at the base of each tank facing the front. After drilling the hole, it was filled with a piece of PVC clear vinyl tubing long enough to bend up to the top edge of the tank and sealed the edge of it with clear silicone waterproof sealant. The opposite end of the tube was then clipped to the top of the tank to keep water from spilling out and plugged to prevent oxygen from entering the bottom of the tank. The sand was then covered with 15 cm of mixed wetland topsoil from four wetlands. Two soils were collected from agricultural drainage treatment wetlands, one of which was the Granada wetland. The third soil was from the Sarita wetland located on the St. Paul campus of the University of Minnesota. The fourth wetland, Reservoir Woods Wetland, was in Roseville, MN, on the northwestern corner of Tamarack Park (44.997636 N, 93.123151

W). All four wetland soils were mixed by hand before being distributed amongst the nine mesocosms.

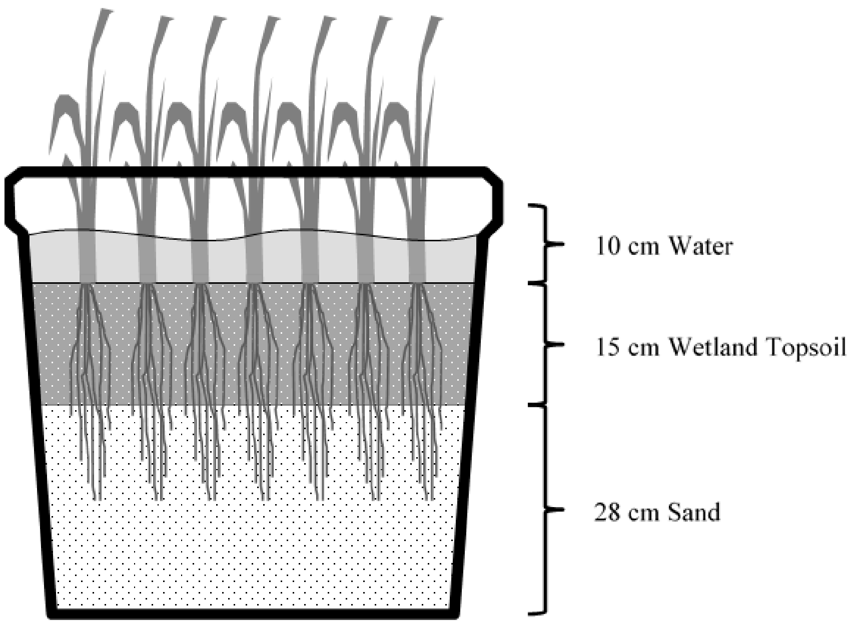

Figure 14. Schematic of the mesocosm setup at the start of each test.

All vegetation was transplanted from the Granada wetland on April 13, 2016 and planted into their respective mesocosms on April 14, 2016. The three plant communities used as the factor levels in this experiment were selected because of their presence in the Granada treatment wetland (Table 21). The three communities were successfully established in the treatment wetland at the time of this experiment. The first cell of the wetland contained a forb-dominant wet prairie mix, the second and third cells were dominated by C4 grasses (mostly switchgrass; *Panicum virgatum)*. Reed canary grass was starting to take over the western edge of each cell.

When planting the vegetation in the mesocosms, the mass of each transplant was kept as similar as possible among tanks. They were also planted in the same arrangement in each tank. Following the transplanting, mesocosms were watered daily for the first few weeks. After this, watering imitated the hydrologic regime in the Granada wetland by inundating the soil once each week and letting it sit until water levels were below the surface. Two switchgrass-dominated tanks were very slow to establish, and some plants within those tanks died. On June 3, 2016, more vegetation from the Granada wetland's cells 2 and 3 was transplanted to these two tanks. These new transplants grew successfully.

Table 21. Species growing in the three plant communities. Each plant community had three replicates.

Wet Prairie Mix[a]	**Switch Grass Mix**[a]	**Reed Canary Grass**[b]
Vernonia fasciculata	*Panicum virgatum*	*Phalaris arundinacea*
Asclepias incarnate	*Carex cf.* Sect. Ovales	
Helenium autumnale	*Leersia oryzoides*	
Physostegia virginiana	*Poa palustris*	
Aster lateriflorum	*Spartina pectinata*	
Carex cf. Sect. Ovales		

[a]Mixes were seeded into the wetland during construction.

[b]Reed canary grass invaded the wetland after construction. No other species were present in the mesocosm tanks.

Nitrate Reductions

At the start of each experiment, the tanks were drained from the basal tube until no water was remaining in each tank. The first two years of pilot studies used concentrations of 24.0 mg L^{-1} NO_3-N, but results from these preliminary experiments indicated that this high of a concentration overwhelms 380-liter mesocosms. Therefore, sodium nitrate pellets were mixed into water in a separate mixing tank until the concentration reached 8.0 mg L^{-1} nitrate/nitrite-N, a low representation of concentrations found in Midwestern tile drainage (Kovacic et al. 1996; Crumpton et al. 2006; Carlson et al. 2013). The mixed nitrate solution was pumped into the surface of each tank using large aquarium pumps and PVC clear vinyl tubing until 10 cm of water covered the soil. Samples were drawn from the drain tube at the bottom of each tank and water flowed out of the tube until the effluent reached approximately 5.0 mg L^{-1} nitrate/nitrite-N or higher. The nitrate concentration was measured using a Hach® Nitratax sc, UV Nitrate sensor.

Nitrate/nitrite-N was measured on the surface and from the bottom drain tube immediately after the tank was done rinsing and filling. The surface nitrate/nitrite-N concentration was measured each of the first three days or until the nitrate concentration stopped rising. Then nitrate was measured on the surface on days 2 or 3, 5, 7 or 8, and 10 following the start of the experiment. Nitrate concentrations were measured from the drainage tube on days 1, 5, and 10 of the experiment. Before reading nitrate from the drainage tube, 200 mL of water was rinsed out of the tube to measure water from the tank rather than the tube. These experiments were repeated five times from November 19, 2016 to January 26, 2017. Dissolved oxygen, oxidation reduction potential, pH, and temperature were also measured on the surface at the same time nitrate was measured using a YSI® Professional Plus Multiparameter Instrument. At the end of the 10th day, pH was also measured from the drain tube at the bottom of each tank. Between each 10-day test, water was drained from each tank and then they were filled again with nitrate water for the next test.

Nitrogen Uptake in Plant Tissue

All above-ground biomass was harvested from each mesocosm on February 2, 2017. Each sample was dried at the same time in an oven set at 60 °C. Below-ground biomass was measured by collecting five 606-cm^3 cores from the same locations in each tank. Root cores were taken from the top 15 cm of soil. The roots and soil were rinsed over multiple sieves to capture all the roots in each core. Roots were removed from the sieves with forceps and pat dried with paper towels. The roots were then weighed and dried at 60 °C. Below-ground biomass harvesting and drying took place from February 3rd to 13th.

After the above and below-ground biomass was dried and weighed, each sample was ground separately using a Thomas® Wiley Mill with a 20-mesh sieve. These dried and ground samples were then analyzed for nitrogen and carbon through a Costech ECS 4010 elemental analyzer using a 3-meter HayeSepQ column. Each sample weighed less than 10 mg and was combusted at 980 °C with the helium gas flow rate set to 110 mol min^{-1} at 1 bar and oxygen gas flow set to 25 ml min^{-1} at 1 bar. Acetanilide was used to generate a standard curve. Combustion analysis yielded a percent nitrogen and percent carbon of the mass of each sample. This percent was used to calculate a C/N ratio in the plant tissue of each sample. The average mass of dry roots in each 606-cm^3 core was extrapolated to represent the 0.138 m^3 of topsoil covering each tank. Due to the experiment occurring after the peak biomass, this experiment most closely reflects a fall drainage event rather than a spring event.

Denitrifying Bacteria Populations

In the fall of 2016, 0.5-g soil samples were collected from the soil in each plant community from the Granada wetland to estimate the population of denitrifying bacteria using qPCR analysis. The soil samples were collected from the same sections of the wetland as the vegetation transplants for the mesocosms. While the transplants were selected from randomized locations, the soil samples were collected in systematic patterns (Figure 15). Samples were immediately placed on ice until they could be placed in the freezer.

The 0.5-g soil samples were thawed, and the DNA was extracted from each sample using the FastDNA SPIN Kit for Soil (MP Biomedicals, LLC) according to the manufacturer's instructions. Following the isolation of DNA, qPCR was used to quantify 16S rRNA genes and fragments of the denitrifying primers (*nosZ*) (Rösch et al. 2002). Standard curves were generated using known quantities of template DNA. The standard curves were linear with an r^2>0.99, and amplification curves were inspected to check for PCR inhibition. The 16S rRNA genes standard concentrations ranged from 1.1 x 10^8 copies μl^{-1} to 1.1 x 10^2 copies μl^{-1}. Negative controls were also included in the qPCR plates that contained water and the reaction mixture instead of DNA solution with the reaction mixture. The reaction mixture in each well of the qPCR plate contained 7.4 µl of DNA-free water, 10 µl of BioRad SYBR Green Master Mix, 1.0 µl of BSA, 0.4 µl of forward primer, and 0.2 µl of reverse primer. The primers had a concentration of 25 µM. A 1.0-µl sample of the unknown DNA solutions, standards, or negatives was added to the reaction mixture in each well. The same volumes of each portion of the reaction mixture were used for the *nosZ* analyses, but the starting quantities of the standards were 4.1×10^8 and 4.0×10^8 copies μl^{-1} for the *nosZ1* and *nosZ2* solutions respectively. The qPCR programs differed for each gene and are described in Table 22. Some of the *nosZ1* samples did not amplify above the detection limit in qPCR, so half of the lower detection limit was used as the value of copies of *nosZ1* for those values for statistical analysis. However, *nosZ2* amplified in all samples and yielded accurate readings of DNA in the samples based on the standard curve.

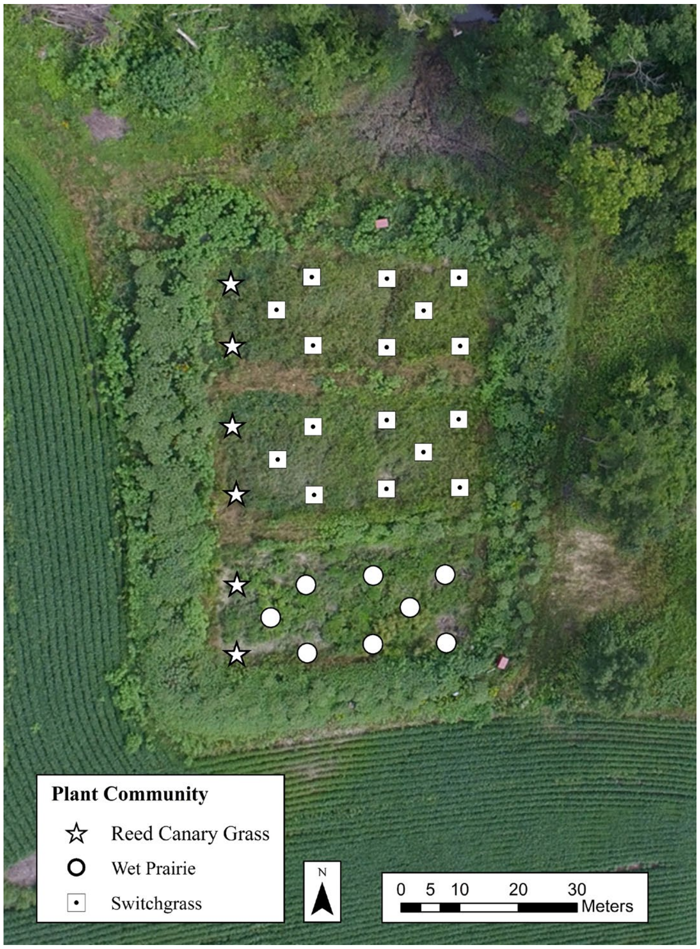

Figure 15. Locations for bacterial samples in the Granada wetland based on the plant communities.

Table 22. Programs used to amplify the target genes in qPCR.

Primers	**Gene**	**Amplicon size (bp)**	**Primer reference**	**qPCR Program**
338F 518R	16S rRNA genes	180	(Bartram et al. 2011)	1 cycle 95℃ 2 min; 40 cycles 95℃ 15 sec, 60℃ 1 min; 1 cycle 95℃ 15 sec; 1 cycle 60℃ 15 sec; Melt Curve: 60-95℃ Increment 0.5℃ 15 sec; 1 cycle 95℃ 15 sec
*nosZ1*F *nosZ1*R	*nosZ*	259	(Henry et al. 2006)	1 cycle 95℃ 2 min; 40 cycles 95℃ 15 sec, 62℃ 1 min; 1 cycle 95℃ 15 sec; 1 cycle 62℃ 15 sec; Melt Curve: 62-95℃ Increment 0.5℃ 15 sec; 1 cycle 95℃ 15 sec
*nosZ2*F *nosZ2*R	*nosZ*	267	(Henry et al. 2006)	1 cycle 95℃ 2 min; 40 cycles 95℃ 15 sec, 54℃ 1 min; 1 cycle 95℃ 15 sec; 1 cycle 54℃ 15 sec; Melt Curve: 54-95℃ Increment 0.5℃ 15 sec; 1 cycle 95℃ 15 sec

Gene abundance was calculated by multiplying the volume of DNA solution in each qPCR well by the total volume of DNA solution created in the extraction process and dividing by the mass of soil used for the extraction (Rosch *et al.* 2002, Rich *et al.* 2003, Baxter *et al.* 2012). These steps were performed with the LaPara Research Group in the University of Minnesota Department of Civil, Environmental, and Geo-Engineering. The 16S rRNA gene primers were used to quantify the total population of bacteria in each soil sample, and the *nosZ* gene was used to quantify the denitrifying bacteria present and the portion of total bacteria that are denitrifiers (*nosZ*:16S rRNA genes).

Statistical Analysis

The mass of nitrate reduced in each tank was calculated by multiplying the change in concentration between the beginning and end of each test in the surface water and the subsurface water by the volume in each soil layer. The subsurface and surface mass reductions were added together to find the total mass reductions. Outliers in the biomass and nitrogen reduction calculations were estimated using the interquartile range method (Tukey 1977). If data were not normally distributed, they were log transformed. All population abundance estimates from qPCR were log transformed before comparing populations. The reductions of nitrate and bacterial populations in the three plant community types were compared using ANOVA, and Tukey's Honestly Significant Difference test was used to assess which communities differed when ANOVA yielded a significant difference.

The other data collected (surface water pH, temperature, dissolved oxygen, and oxidation reduction potential) were analyzed using MANOVA having vegetation community type as the independent factor. These were analyzed to determine if there was a relationship with the measured reduction of nitrates among the plant communities. Some variables also needed to be transformed to display normal distribution. Tukey's honestly significant difference was used to assess which treatment means differed when MANOVA yielded a significant difference. The soil DNA samples were also grouped again by wetland cell instead of plant community to determine if cell location was a confounding variable in bacterial populations.

Results

Nitrate Reductions

The wet prairie mix tanks removed an average of 124 mg and 171 mg more total mass of nitrate-N than the reed canary grass and switchgrass tanks in each test, respectively ($p = 0.08$ & 0.01). Switchgrass and reed canary grass communities did not differ significantly in total reductions ($p = 0.67$). On average, the wet prairie mix tanks removed over 225 mg more nitrate-N from the surface water than the other communities' tanks ($p < 0.01$). However, the reed canary grass tanks removed an average of over 70 mg more nitrate-N from the subsurface water than the other plant communities' tanks ($p < 0.01$, Figure 16).

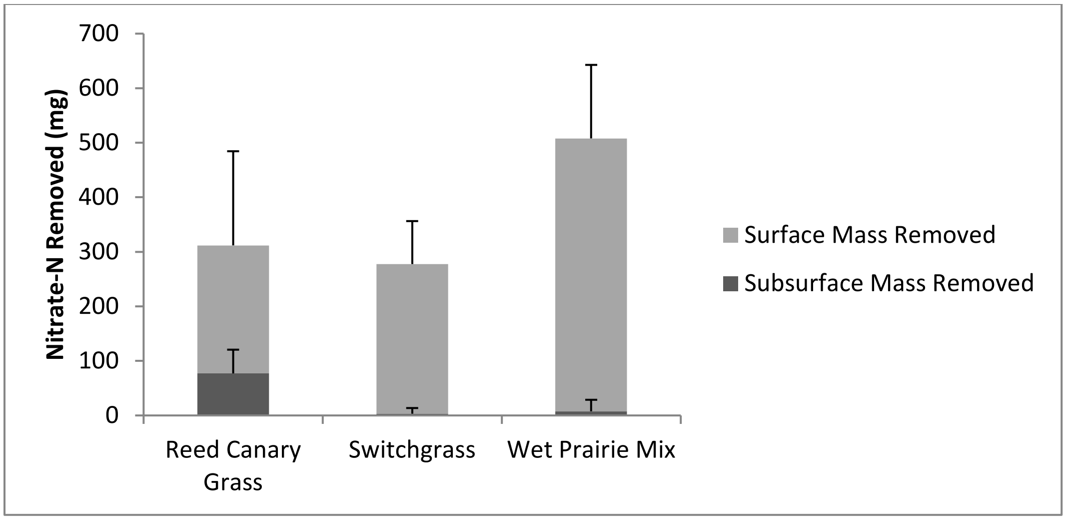

Figure 16. Mean mass of nitrate-N removed by each plant community. Error bars are the standard deviation for surface and subsurface removal.

Among the surface water temperature, dissolved oxygen, pH, and oxidation reduction potential parameters measured, only dissolved oxygen and pH showed significant differences among plant communities. The wet prairie mix and reed canary grass mesocosms had significantly different average dissolved oxygen concentrations of 3.8 and 5.8 mg L^{-1} respectively (Table 23; $p = 0.03$), and wet prairie mix and switchgrass mix mesocosms had significant differences in average pH of 7.19 and 7.31 respectively ($p = 0.006$). However, dissolved oxygen was the only parameter to display a significant linear relationship with the surface nitrate reductions ($y = -8.0056x + 773.83$, $R^2 = 0.4$, $p < 0.001$; Figure 17).

Table 23. Parameter averages from each test. Standard deviation of the averages from each test is in parentheses.

Plant Community	Temperature (°C)	Dissolved Oxygen (mg L^{-1})	pH	ORP (mV)
Reed Canary Grass	15.7 (1.0)	5.8 (1.03)	7.24 (0.09)	97.3 (9.0)
Switchgrass Mix	16.2 (0.9)	6.3 (0.8)	7.31 (0.11)	98.0 (9.1)
Wet Prairie Mix	15.8 (0.9)	3.8 (0.8)	7.19 (0.10)	101 (9.7)

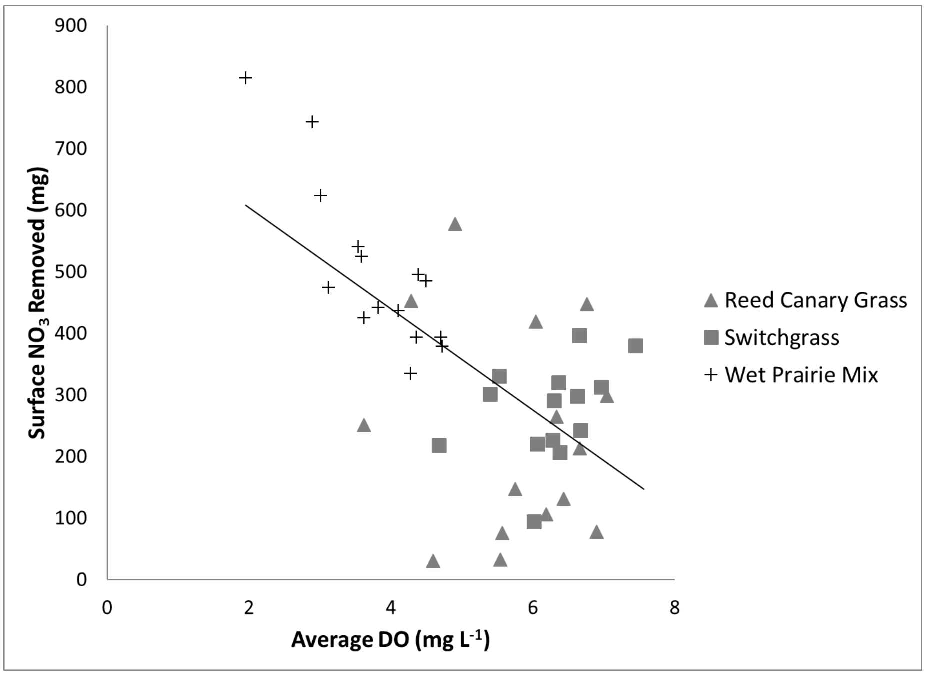

Figure 17. Relationship between the dissolved oxygen (DO) on the surface of each tank and the nitrate removed on the surface in each experiment.

Nitrogen Uptake in Plant Tissue

Reed canary grass had a greater average root mass than both the switchgrass and wet prairie mix ($p = 0.001$ & $p < 0.001$ respectively, Table 24). The switchgrass mix mesocosms had an average above-ground biomass that was significantly lower than the reed canary grass tanks' average ($p = 0.08$, Table 24).

Table 24. Average dry weight biomass of each plant community per mesocosm.

Plant Community	Below-Ground Biomass (g)	cv	Above-Ground Biomass (g)	cv	Estimated Below-Ground Biomass in Transplants (g)	cv	Total Biomass Growth Since Planting (g)	cv
Reed Canary Grass	401	0.86	209	0.45	62.5	0.18	548	0.28
Switchgrass Mix	287	1.55	70.9	0.26	48.9	0.98	309	0.93
Wet Prairie Mix	349	2.08	190	0.29	45.0	0.59	495	0.55

There was no significant difference among the plant communities' nitrogen assimilations except between the switchgrass mix and reed canary grass above-ground nitrogen assimilations (Table 25, $p=0.04$). The C/N ratio for above-ground tissue was significantly lower in the reed canary grass than the other plant communities ($p < 0.04$, Table 26 & Figure 18), and the C/N ratio for below-ground tissue was significantly lower in the wet prairie mix than the other plant communities ($p < 0.05$, Figure 18).

Table 25. Average nitrogen and carbon mass composition measured through combustion analysis of plant tissue samples in each plant community.

Mesocosm	Nitrogen in Below-Ground Tissue (%)	cv	Nitrogen in Above-Ground Tissue (%)	cv	Carbon in Below-Ground Tissue (%)	cv	Carbon in Above-Ground Tissue (%)	cv
Reed Canary Grass	1.3	0.066	2.1	0.062	42.8	0.024	41.3	0.015
Switchgrass Mix	1.1	0.16	1.3	0.17	38.5	0.14	42.6	0.052
Wet Prairie Mix	2.2	0.68	1.3	0.020	41.5	0.058	41.0	0.050

Table 26. Estimated average mass of nitrogen (N) and carbon (C) in the plant tissue of each plant community.

Mesocosm Label	Above-Ground N (g)	cv	Below-Ground N (g)	cv	Total N (g)	Above-Ground C (g)	cv	Below-Ground C (g)	cv	Total C (g)	Above-Ground C/N ratio	Below-Ground C/N ratio
Reed Canary Grass	4.44	0.49	5.21	0.19	9.65	86.1	0.44	171	0.17	257	19.8	33.0
Switchgrass Mix	0.910	0.30	3.12	1.12	4.03	30.5	0.30	100	1.02	130	33.8	35.8
Wet Prairie Mix	2.51	0.29	13.7	1.02	16.2	78.2	0.31	149	0.73	227	31.1	15.0

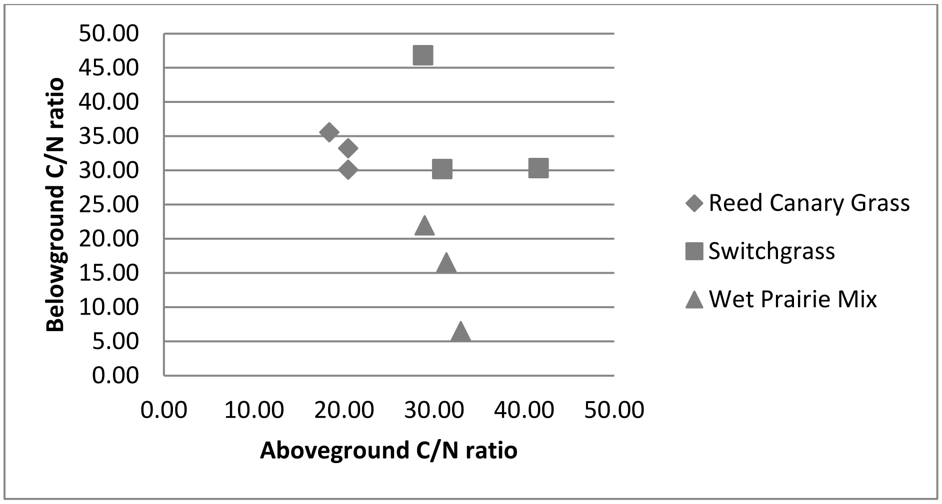

Figure 18. C/N ratio in belowground and aboveground tissue in each mesocosm.

Denitrifying Bacteria Populations

The number of copies of 16S rRNA genes per gram of soil ranged from 1.3 x 10^6 to 6.4 x 10^9 throughout the wetland in 2016, and the copies of the *nosZ1* and *nosZ2* gene per gram of soil ranged from 1.2 x 10^5 to 2.5 x 10^7 and 1.2 x 10^5 to 8.8 x 10^8, respectively. However, there were no significant differences in the abundances of 16S rRNA genes or *nosZ* genes between the plant communities. The *nosZ2*:16S rRNA genes ratios ranged from 0.03 to 0.21 for the reed canary grass, 0.03 to 0.19 for the switchgrass mix, and 0.10 to 0.18 for the wet prairie mix communities. The ratios were significantly higher in the root zone of the wet prairie mix than in the root zone of the reed canary grass and switchgrass communities ($p = 0.03$ & 0.05, Figure 19). Therefore, the portion of bacteria in the wetland that were denitrifiers was greater in the wet prairie mix root zone than the other plants' root zones. The ratio did not differ significantly among the cells and there was no interference between cell location and vegetation.

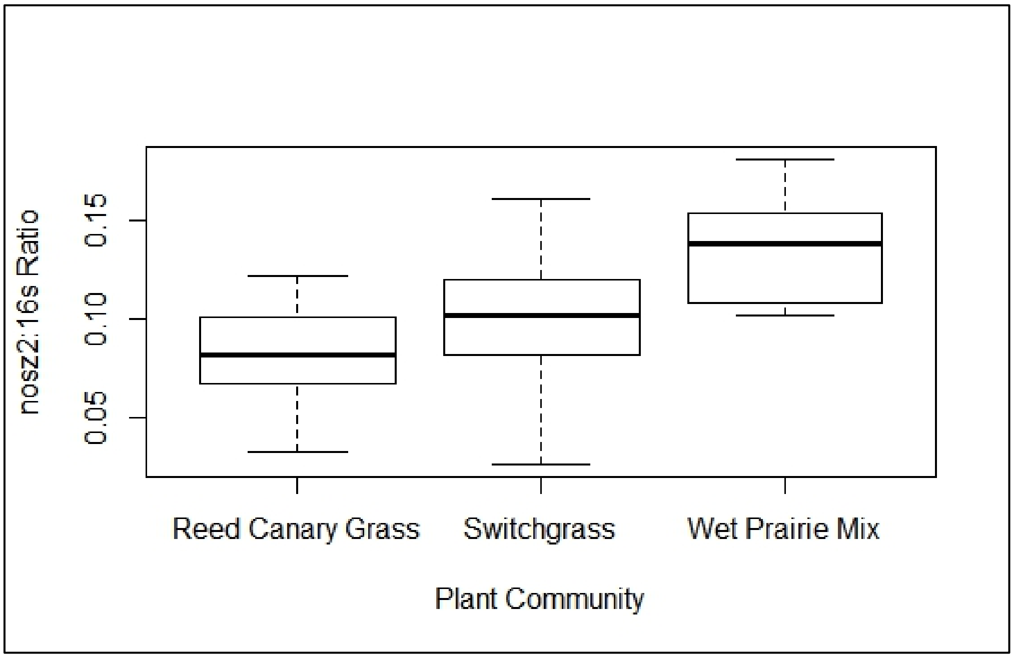

Figure 19. Ratio of *nosZ2*:16S rRNA gene copies from the plant communities.

Discussion

The hypothesis was that there would be a significant difference in the reduction of nitrate between reed canary grass and the native plant communities. The wet prairie mix removed the most nitrate from the surface of each mesocosm ($p < 0.01$), reed canary grass removed the most below the surface ($p < 0.01$), and the wet prairie mix removed the most nitrate in total ($p = 0.08$). Therefore, the hypothesis was supported.

These results suggest that wet prairie mixes similar to this mix are likely to reduce more nitrate in edge-of-field treatment wetlands than reed canary grass. While the results from this project indicate that one plant community may be better than another at removing nitrate from tile drainage, there is still uncertainty as to why that would be the result. The first possible explanation for the differences in reductions among plant communities was the poor establishment of switchgrass. There was a possibility that switchgrass could have had the greatest biomass of any of the plant communities due to the well-established switchgrass tank having the greatest growth of all the tanks. However, poor switchgrass growth in the other two tanks led to a mix of other species growing more in those tanks that may not have represented typical switchgrass communities. This poor switchgrass growth and shift in species may have impacted the nitrate removal.

The C/N ratio could have revealed another influence on denitrification. The C/N ratio was lowest in the below-ground wet prairie mix tissue. For above-ground tissues, the C/N ratio was lowest in the reed canary grass. The wet prairie plant species flowered and began senescing much earlier than the grass species in the other tanks. Much of the tissue in the reed canary grass was still green at the time of harvest following the experiment. The C/N ratio tends to be higher in dead plant material than living tissue (Cross et al. 2005). Therefore, the C/N ratio in the above-ground tissue was higher in the wet prairie species because they were already senesced. Conversely, reed canary grass was still assimilating nutrients into its above-ground tissue later in the season. However, the mass of nitrogen assimilated was still less than what was denitrified.

A third potential reason for variation in nitrate removal was the relationship between dissolved oxygen and nitrate removal. Denitrification has been observed when dissolved oxygen was above 4.0 mg L^{-1} (Lin et al. 2002), but it typically needs to be below 0.3–1.5 mg L^{-1} (Kadlec and Wallace 2008; McAllister 1993) or 1.9-4.0 mg L^{-1} (Tesoriero and Puckett 2011) in order for denitrification to occur. The surface concentrations were above these limits throughout the experiment in most of the tanks. However, some bacterial species are adapted to use nitrate for denitrification in the presence of oxygen (Jones et al. 2013; Ligi et al. 2013). Some species even prefer denitrification over oxygen respiration, so denitrifiers can quickly switch from oxygen to nitrate when oxygen is being depleted in these communities (Miyahara et al. 2010). Nonetheless, the concentrations below the soil surface were likely much lower than the surface concentrations (Crumpton and Phipps 1992; Kadlec and Wallace 2008).

While reed canary grass tanks had a greater concentration of dissolved oxygen than the wet prairie mix, they also had the greatest reduction of nitrate below the surface and the greatest belowground biomass. In contrast, the wet prairie mix had the lowest concentration of dissolved oxygen with the greatest reduction of nitrate on the surface. The latter correlation is the more common scenario considering denitrification requires oxygen-limited conditions, but the former could be due to nitrogen assimilation still occurring through the roots and reed canary grass oxygenating the rhizosphere (Edwards et al. 2006; Jørgensen, Struwe, and Elberling 2012). While the wet prairie plants had begun to senesce by the time the tests began, the reed canary grass was likely still assimilating nitrogen. Furthermore, root exudates have also been shown to impact microbial enzymatic activities, including denitrification (Henry et al. 2008; Mounier et al. 2004; Philippot et al. 2007). Perhaps a combination of oxygen and exudates being released in the rhizosphere of the reed canary grass slowed microbial decomposition, but measuring this effect was beyond the scope of this study.

Another potential explanation for the variation in nitrate removal in the mesocosms was the denitrifying microbe abundance in each root zone. Although the abundance of bacteria and denitrifiers was not significantly different among the soils of each plant community, the ratio of *nosZ2* gene copies to 16S rRNA gene copies was significantly greater in the rhizosphere of the wet prairie mix than the reed canary grass or switchgrass mix. The microbial community in the wet prairie mix seemed to be adapting to the conditions in the wetland better than the other communities. Because the nitrate removal was greatest in the wet prairie community mesocosms, the adapted communities performed better in this experiment.

The 16S rRNA gene copies g^{-1} ranged from 1.3×10^6 to 6.4×10^9 at the Granada wetland. These abundances are comparable to those of other studies which quantified the same genes in various soil types (Table 27). Wang et al. (2013) measured an average 16S rRNA gene abundance in the wet soils of 5.5×10^{10} gene copies g^{-1} dry weight (2.0 times lower than the average of the drier soils they sampled). Their average abundance of *nosZ* genes in the wet soils was 1.4×10^9 gene copies g^{-1} dry weight (2.6 times lower than the drier soils).

Table 27. Abundances of 16S rRNA genes and *nosZ* from other studies.

Gene	Mean (copies g^{-1} dry weight)	Range (copies g^{-1} dry weight)	*nosZ*:16S rRNA genes (%)	Soil Type	Reference	Location
16S rRNA		1.8×10^9 - 1.2×10^{10}	0.17 – 0.69	Riparian Wetlands	(Ligi et al. 2013)	Columbus, OH
nosZ		4.9×10^6 - 7.0×10^7				
16S rRNA	5.5×10^{10}	max = 3.1×10^{11}	~2.5	Littoral Zone	(Wang et al. 2013)	Baiyangdian Lake, China
nosZ1	1.4×10^9	max = 1.0×10^{10}				
16S rRNA		0.30×10^7 - 8.7×10^7		Stream Sediment	(Baxter et al. 2012)	Indiana
nosZ		0.20×10^6 - 1.8×10^6				
16S rRNA		10^8 - 10^{10}		Constructed wetland microcosms	(Chen et al. 2014)	China
nosZ2		0.50×10^8 – 5.0×10^8				
16S rRNA		~5×10^8 – 10^9	0.2 - 5	Agricultural Soil	(Henry et al. 2006)	France
nosZ1 & *nosZ2*	~10^7					
16S rRNA	~5.0×10^8		0.5	Wetland Marsh	(Henry et al. 2006)	Slovenia
nosZ1 & *nosZ2*	~10^6					
nosZ		~1×10^5 – 4×10^5		Pothole Wetlands	(Ma et al. 2008)	Saskatchewan, Canada
16S rRNA	3.3×10^8	1.3×10^6 – 6.4×10^9		Constructed Wetland	This study	Granada, MN
nosZ1	1.2×10^6	1.2×10^5 – 2.5×10^7	2.5			
nosZ2	4.4×10^7	1.2×10^5 – 8.8×10^8	11			

The relative abundance of *nosZ* genes to 16S rRNA genes in agricultural soils ranged from 0.1 to 5% of 16S rRNA genes (Henry et al. 2006). The relative abundance in wetland soil of the same study was approximately 0.5% (Henry et al. 2006). In wetlands from other studies, these ratios seemed to be consistently under 3%, whereas the relative abundance of *nosZ2* to 16S rRNA genes in the wetland soils of this study ranged from 1 to 12% (Henry et al. 2006; Tiedje 1988). The proportions of *nosZ1* to 16S rRNA genes were much lower and ranged from 0.1 to 3.2%. It is unknown why the *nosZ2* abundances were greater than the *nosZ1* abundances in this study, but both ratios exceeded those in other studies. Ligi et al. (2013) noted that proportions of denitrifying bacterial communities are impacted by nitrate concentrations. Therefore, the high nitrate concentrations in the Granada wetland could have been one of the primary reasons the *nosZ*:16S rRNA genes ratios were greater than those of other studies.

Conclusion

Nitrate removal was greater in mesocosms planted with a wet prairie mix than with reed canary grass. The greater removal by the wet prairie mix was mainly due to a lower dissolved oxygen concentration in the wet prairie mix tanks and a greater ratio of denitrifying bacteria to total bacteria (*nosZ:*16S rRNA genes) in the wet prairie mix root zone. The mesocosm tanks planted with switchgrass did not establish well and, therefore, did not perform well in the nitrate removal experiments.

Our experiment suggests that plant community types can affect the nitrate removal rate in tile drainage treatment wetlands. In this case, reed canary grass invasions in treatment wetlands could decrease the effectiveness of those wetlands. Although the plant communities did not impact the total populations of bacteria in the rhizosphere, they did impact the portion of bacteria adapted for denitrification.

Chapter 3: Modeling the applicability of edge-of-field treatment wetlands to reduce nitrate loads in the Elm Creek, Minnesota watershed

Abstract

Constructed agricultural treatment wetlands are key tools for removing nitrate from surface waters. Due to limited funds for nitrate removal practices, investments need to be in the most cost-effective practices. Furthermore, nitrate removal practices that take less land out of production may be more appealing for farmers. Therefore, small, edge-of-field wetlands with drainage areas covering fewer than 60 ha were compared to large wetlands with drainage areas greater than 60 ha to determine each size's nitrate removal effectiveness and cost effectiveness. The Agricultural Conservation Planning Framework toolbox model was used to determine best placements for each wetland type in the Elm Creek watershed in southern Minnesota. The Soil and Water Assessment Tool model was used to estimate the volume of tile discharge and nitrate-nitrogen concentration into each wetland over a 10-year period. A spreadsheet model was used to estimate the reductions of nitrate in each wetland over the same 10-year period. Small, edge-of-field wetlands with a saturated hydraulic conductivity (Ksat) of 0.17 m day^{-1} were more effective at removing nitrate for the area removed from production (kg ha^{-1} year^{-1}) and as cost effective ($ kg^{-1} nitrate-N removed) as the large wetlands. When the small wetlands had a low Ksat (8.64×10^{-5} m day^{-1}), they were more effective for each area removed from production ($p = 0.06$) but not as cost effective ($p = 0.003$). This study suggests that constructing many small, edge-of-field treatment wetlands with high conductivity to

reach nutrient reduction goals would cost the same as constructing large wetlands but would remove fewer hectares of cropland from production.

Introduction

Intensive research has been necessary to better understand how various technologies and practices will help reduce nutrient pollution. As field and laboratory studies evaluate the effectiveness of individual best management practices (BMPs) under specific conditions, modeling studies are necessary for understanding the potential impact of the practices at a watershed scale. Numerous models have been utilized in Minnesota to predict the reductions of nitrogen and phosphorus using various agricultural BMPs compared to current land use practices. These models vary from spreadsheet calculators to GIS toolboxes. In agricultural landscapes, some of the common models include the Phosphorus Index (P-Index; Lewandowski et al. 2006); Agricultural Policy/Environmental eXtender (APEX; Jeong et al. 2015); Agricultural Conservation Planning Framework (ACPF; Porter et al. 2016); Soil and Water Assessment Tool (SWAT; Arnold et al. 2012); and the Prioritize, Target, Measure Application (PTMApp; Houston Engineering Inc. 2016). Many of the BMP tools and models are gradually adding practices as improved technologies and datasets are becoming available.

While many practices can be included in these models, not many have the ability at this time to model small, edge-of-field tile treatment wetlands. Some of the common edge-of-field practices in these models are bioreactors, saturated buffers, restored wetlands, constructed wetlands, and impoundments. However, none of these practices perfectly match what is needed in a model for the assessment of small, edge-of-field treatment wetlands across a watershed. In many of these models, there are options for simulating the potential nutrient reductions of large, restored wetlands within watersheds. For example, the ACPF tool can produce maps of ideal placement locations for nutrient reduction wetlands within a given watershed. However, this model requires a minimum watershed of 60 ha for it to recognize the watershed as an ideal location for a nutrient reduction wetland. The SWAT model can model the impact of wetlands on water quality, but it lacks some of the abilities to model the ideal placement of small, tile drainage treatment wetlands at this time. Other models, like APEX, work best when rich amounts of crop field data and management history are available as inputs for a specified watershed.

Now that a small, edge-of-field wetland has been thoroughly studied for its effectiveness at reducing nutrients from tile discharge in Minnesota (Lenhart et al. 2016), and other similar wetlands have been studied in other parts of the Midwest (Larson et al. 2000; Mitsch, Day, et al. 2005; Phipps and Crumpton 1994), questions should be asked regarding where it fits in nutrient reduction strategies. There are Natural Resources Conservation Service (NRCS) programs – such as the Conservation Reserve Program, Environmental Quality Incentives Program, or Agricultural Conservation Easement Program – from the Farm Bill which can fund the construction of these small wetlands (Lucas 2014). States also have funds for the restoration of wetlands which could be used for these smaller designs. However, it is helpful for decision makers to know how a wetland less than 0.5 ha in size can be scaled up to reduce the nitrate load in the Mississippi River.

Mitsch et al. (2001) estimated that 22,000 km^2 of restored wetlands are required to reduce the load of nitrogen in the Mississippi River by 40%. Therefore, organizations are attempting to restore larger wetlands such as the 2,800-ha Emiquon floodplain in Illinois by The Nature Conservancy or 1,400-ha Tensas River project in Louisiana in order to reduce larger loads of nitrogen in each restoration project (Hunter and Faulkner 2001; TNC 2016). The Iowa Conservation Reserve Enhancement Program (CREP) requires that sites submitted for funding have a minimum watershed area of 200 ha (IDALS 2016a). While these larger wetlands produce greater reductions in a single project, they also require more funding for each project and oftentimes more landowners who are willing to collaboratively sell their land.

Other BMPs such as bioreactors cover less than 0.1 ha and are underground, so they take negligible amounts of land out of production and are successfully being constructed throughout the Midwest to remove nitrate from tile drainage (Addy et al. 2016; University of Illinois 2018). These smaller practices can be effective and impactful when installed on enough properties. Therefore, models need to be utilized to determine how to scale up the impact of small, edge-of-field treatment wetlands to reduce similar loads to those reduced by larger wetlands. Furthermore, their cost effectiveness needs to be analyzed. There is limited federal and state funding for the construction of the number of BMPs needed to reach nitrogen reduction goals. Therefore, the most cost-effective practices should be prioritized when investing funds.

The objectives of this study were to utilize currently available models to 1) map potential locations for edge-of-field tile treatment wetlands in the HUC-12 watershed where the Granada wetland from the previous study was located (Lenhart et al. 2016), 2) model hydrologic inputs for tile drainage at each modeled location, 3) estimate potential nitrate reductions of wetlands at each location, and 4) compare the modeled reductions and costs of these wetlands to those of larger treatment wetlands in the same watershed. The first hypothesis for this study is that there will be no significant difference between the reduction of nitrate per unit area converted for small wetlands versus large wetlands. The second hypothesis is that the cost per mass of nitrate removed will not be significantly different between the two wetland sizes.

Methods

The HUC-12 Elm Creek watershed, where the study site is located, received funding through the NRCS National Water Quality Initiative as a high priority watershed and is impaired for aquatic life, aquatic recreation, turbidity, and high level of *E. coli* (Minnesota USDA-NRCS 2015; MPCA 2016a). The Granada wetland is a small, edge-of-field treatment wetland located in Martin County 6.9 km north of Granada, MN. The wetland was designed in 2012 and constructed in the spring of 2013 (Karlheim 2012; Lenhart et al. 2016). Approximately 10.1 ha of subsurface tile drainage was rerouted to flow into the wetland.

The entire Elm Creek watershed resides within the area impacted by the Des Moines Lobe, an area formed from a portion of the most recent glacier in Minnesota

(Bettis, Quade, and Kemmis 1996; Prior 1991). The landscape has many small hills and ridges with gently undulating slopes and broad lowland areas (Matzdorf 1989). The Canisteo-Clarion, Canisteo-Glencoe, and Clarion-Delft-Storden associations are the most common in this watershed, and poorly drained Coland loams are the dominant soil in the floodplains of Elm Creek. Many of the slopes and slope crests are well drained and some pockets of sandy outwash are found in historic and current stream channels, but the flat areas, depressions, and floodplains are poorly drained. Due to the latter areas being the most common, much of this watershed is tile drained and farmed (Matzdorf 1989).

Due to the availability of many models but limitations within each for modeling small, edge-of-field tile treatment wetlands, multiple models were used collaboratively for this study. The first objective of the study was accomplished using the ACPF tool in the predefined HUC-12 Elm Creek watershed. The ACPF tool estimates ideal locations for nutrient removal wetlands using ArcGIS®. However, the minimum watershed for nutrient removal wetlands is too large for the treatment wetlands in this study because the wetland modeling component of the tool is made to imitate CREP wetlands in Iowa (Crumpton and Stenback 2016). The tool was used for mapping edge-of-field bioreactors instead. Bioreactors are designed to treat watersheds ranging from 8 to 40.5 ha in this model, and they utilize the model's predictions for locating tile-drained fields (Porter et al. 2016). Their placements along field edges, treatment of tile drainage, and ideal slope match those of edge-of-field wetlands as well. Therefore, the step-by-step process in the ACPF tool handbook for optimal bioreactor sites (Porter et al. 2016) was used to create a map of the Elm Creek watershed with a polygon output locating all the bioreactor locations and watershed sizes for each bioreactor. More methods regarding the ACPF toolbox are described in Appendix 3.

The second objective of this study – to model hydrologic inputs for tile drainage at each modeled wetland location – was accomplished using SWAT. Although SWAT is an extensive model with many capabilities for estimating the nutrient reductions from installing various BMPs, it has not yet been designed to assess edge-of-field, tile treatment wetlands. However, it is a model oftentimes used for creating hydrographs over multiple years for a designated field or watershed. It utilizes soil, weather, slope, acreage, land use, and other parameters for estimating watershed boundaries and hydrology of the selected watershed. The other parameters included inputs such as drain tile depth and the depth to an impervious layer. Measured tile discharge and weather from the Granada wetland were used to calibrate the model along with weather data from the Fairmont Airport approximately 11.5 km southwest of the Granada wetland. Other descriptions of SWAT inputs are in Appendix 3.

Following the calibration and validation of each site, the watersheds were modeled for each wetland site. Each watershed had daily hydrology and nitrate outputs for water discharging from the watershed's tile outlet where the wetland would be constructed. The outputs were modeled for 2007-2016 to model tile drainage responses to 10 years of measured weather data rather than using predicted weather.

To accomplish the third objective – estimate the nutrient reduction potential of each wetland –the daily discharge, nitrate load, and evapotranspiration from each drainage area were inputs in a spreadsheet model used by Lenhart et al. (2016). The model was created in Microsoft® Excel to calculate the daily water budget and nitrogen removal based on wetland size, weir size, daily precipitation, tile discharge, tile nitrate concentration, evapotranspiration, vertical saturated hydraulic conductivity, and the nitrate reaction coefficient. The spreadsheet model used Equation 15 below to determine the daily flow out of the wetland (Karlheim 2012).

Equation 15:

$$Q_o = C_E * W_w (H_o - H_w)^{1.5}$$

Q_o = outflow rate (m^3 day^{-1})

C_E = weir discharge coefficient (m^3 day^{-1}) ($m^{2.5}$)

W_w = width of weir (m)

H_o = water surface elevation at wetland outlet (m)

H_w = weir crest elevation (m)

The size of each wetland was designed to be 1% of the drainage area to keep each wetland less than 0.5 ha and make the wetland-to-watershed area ratio like the Granada wetland's ratio. The outlet weir height was 0.9 m and buffer height was 1.5 m to match those from the ACPF tool's modeling specifications. The outlet weir width was adjusted for each wetland's spreadsheet model until the maximum water depth in the wetland was 1.0 m to prevent water from flooding over the 1.5-m buffers. The model was calibrated using measured nitrate removal and the water budget in the Granada wetland from 2014 through 2016. When calibrating, a vertical saturated hydraulic conductivity value (Ksat) was calculated to match the wetland's water budget. The calibration resulted in a Ksat of 0.17 m day^{-1} (±0.10) in the Granada wetland, which was very close to the measured 0.19 m day^{-1} using infiltrometers at the wetland. This is higher than recommended in USDA NRCS practice standards for constructed surface flow wetlands (USDA NRCS 2009, 2018). Therefore, both the recommended Ksat value of 8.64×10^{-5} m day^{-1} and the 0.17 m

day^{-1} from the spreadsheet calibrations were used to estimate nutrient reductions in each wetland for the third objective. The nitrate reaction coefficient from the Granada wetland's shallow groundwater was also used to estimate nitrate removal in water that infiltrated from the surface of each wetland into the shallow groundwater.

The mass of nitrate-nitrogen (nitrate-N) removed from all the wetlands over the modeled 10-year period were added together to calculate a potential maximum mass of nitrate-N that could be removed using small, edge-of-field wetlands throughout the HUC-12 Elm Creek watershed. This sum was divided by the total area that would be converted to these wetlands to estimate the wetland reduction efficiency. The land converted to these wetlands included the buffer surrounding the ponded treatment area. Present value costs of wetland construction were then used to estimate the cost for each kilogram of nitrate-N removed (Table 28). Although inflation and lost crop income could also be considered (Crumpton et al. 2008; Prato et al. 1995), they were beyond the scope of this project.

Table 28. Costs used for each wetland based on estimates from Christianson et al. (2013).

Item	Cost	Notes	Reference
Control Structure	\$602.81 - 1,615.27	Size and cost dependent on wetland size	(Agri Drain Corporation 2018)
Engineering Design	\$28,800	Assuming \$40 hr^{-1}, 8 hr day^{-1}, 15 days	(Christianson et al. 2013)
Contractor/ Construction Fees (ha^{-1})	\$1,076.87	\$161.45 hr^{-1} at 6.67 hr ha^{-1}	(Extension 2018)
Seeding and Seed Mix (ha^{-1})	\$344.61	CRP Economy Seed Mix	(Extension 2018; Prairie Land Management 2005)
Land Acquisition (ha^{-1})	\$12,973.03	Martin County, MN average land value	(AcreValue - Granular Inc. 2018)
Maintenance (ha^{-1})	\$44.23	Spot mowing	(Extension 2018)

The final objective of this study, to compare small, edge-of-field wetlands to larger wetlands, was accomplished by running the ACPF tool for nutrient reduction wetlands. Wetland locations and drainage areas were estimated using the ACPF tool for these larger wetlands (drainage areas > 60 ha). The drainage areas of each wetland were then modeled in SWAT to estimate discharge, nitrate loads, and evapotranspiration from these areas similarly to the drainage areas for the smaller wetlands. Eleven wetland sites were modeled in ACPF, but two were combined because they overlapped and because the SWAT model was unable to distinguish their drainage areas. Because these wetlands in the ACPF tool were sited to treat areas with tile drainage, the same tile drainage input parameters were used in these watersheds as in the small wetland watersheds.

After running the SWAT model, the same spreadsheet model used to estimate nitrate removal in the small wetlands was used to estimate nitrate reductions in the large wetlands. Only the low Ksat value was used for the large wetlands due to the overwhelming infiltration volumes resulting from a large Ksat value. The total modeled reductions using larger wetlands were added together. The removal effectiveness was calculated by dividing the mass of nitrate-N removed by the hectares of wetland and buffer constructed. The costs were calculated with the same values as those used for the small wetlands (Table 28) and then divided by the kilograms of nitrate-N removed to calculate the cost effectiveness. The removal and cost effectiveness of the small wetlands were compared to those of the large wetlands. The effectiveness data were not normally distributed, so the two-sample Mann-Whitney U test was used in Rstudio® to calculate the mean differences.

Results

The ACPF tool identified 65 ideal locations for small, edge-of-field tile drainage treatment wetlands. The watershed area for each wetland ranged from 8.4 to 48.0 ha. Three watersheds had more than one possible wetland site at the outlet due to the model finding two options for wetland placement within each of these watersheds. After duplicates were removed, there were 62 locations remaining (Figure 20 & Table 42).

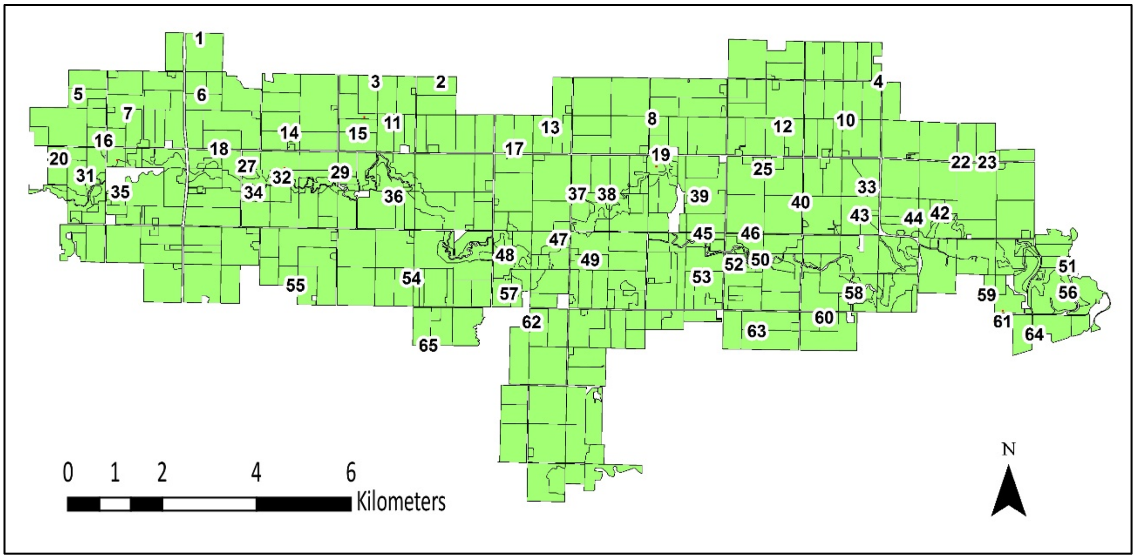

Figure 20. Map of the best locations for small, edge-of-field tile drainage treatment wetlands from the ACPF tool.

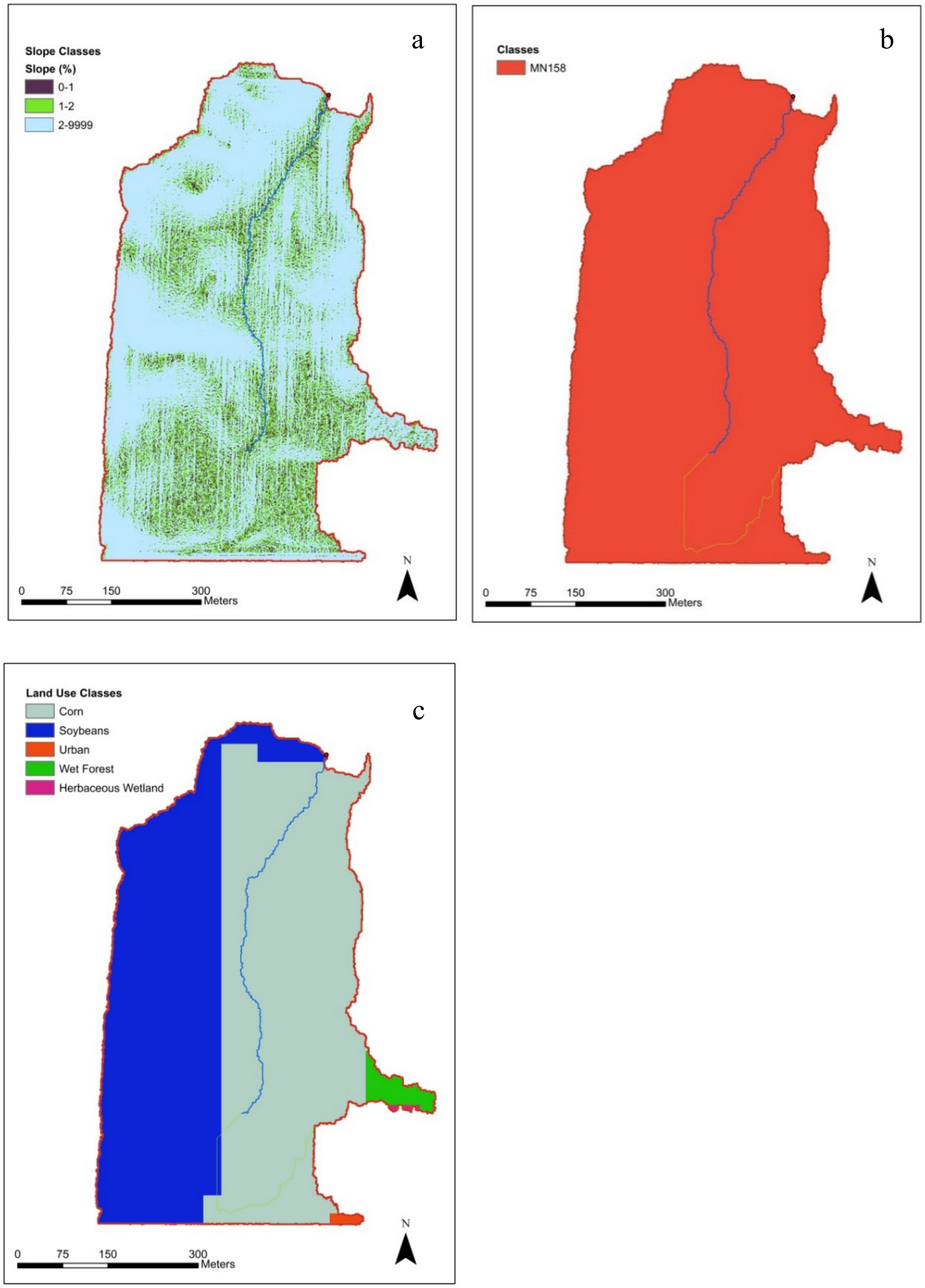

Figure 21. Example of three SWAT layers for one wetland drainage area, a) slope class layer, b) soil class layer, and c) land use class layer.

The SWAT model predicted that the nitrate-N mass discharge from each watershed (Figure 21) of the small wetlands ranged from 23.10 to 1,181 kg yr^{-1} over the course of 10 years and averaged 292.9 kg yr^{-1} among the 62 wetlands. Approximately 18,160 kg nitrate-N yr^{-1} were discharged from all 62 drainage areas, which totaled 1,192 ha (Table 42).

Due to the variability in wetland and watershed size, as well as variability in nitrate loads discharging into each wetland, nitrate load reductions varied among the small, edge-of-field wetlands. Percent removal in the wetlands with a Ksat of 8.64×10^{-5} m day^{-1} (low-conductivity wetlands) averaged 19.1% over 10 years, and removal in the wetlands with a Ksat of 0.17 m day^{-1} (high-conductivity wetlands) averaged 66.2% over 10 years. The mean wetland removal rate was 163 kg ha^{-1} yr^{-1} for low-conductivity wetlands and 488 kg ha^{-1} yr^{-1} for the high-conductivity wetlands. In terms of total mass removed per year, this is approximately 3,510 to 12,500 kg yr^{-1} of the 18,200 kg yr^{-1} discharging from all 62 watersheds. The cost of each wetland over the course of 10 years averaged \$35,280.00 (SD = \$3,016.00). Therefore, the mean cost effectiveness for the low- and high-conductivity wetlands were \$18.49 kg-N^{-1} yr^{-1} and \$5.02 kg-N^{-1} yr^{-1}, respectively (SD = \$22.59 kg^{-1} & \$5.55 kg^{-1}, respectively). The cost effectiveness of all 62 wetlands combined was \$6.24 and \$1.76 for the low- and high-conductivity wetlands, respectively.

The total watershed area of the 10 larger wetlands was greater than the total drainage area of the edge-of-field wetlands with 3,248 ha draining into the larger wetlands but only 1,192 ha draining into the smaller wetlands (Figure 22). The average size of the wetland and buffer of the larger wetlands was 11.9 ha. From the 3,248-ha total drainage area, 28,040 kg nitrate-N were discharged from tile drainage each year (Table 43). The estimated nitrate-N removal rate was 6,340 kg yr^{-1} (22.6%). The removal effectiveness of these wetlands was then 80.1 kg ha^{-1} yr^{-1}. This was a lower removal effectiveness than the smaller wetlands' on a per unit area basis (163 & 488 kg ha^{-1} yr^{-1}; $p = 0.06$ & $p < 0.001$).

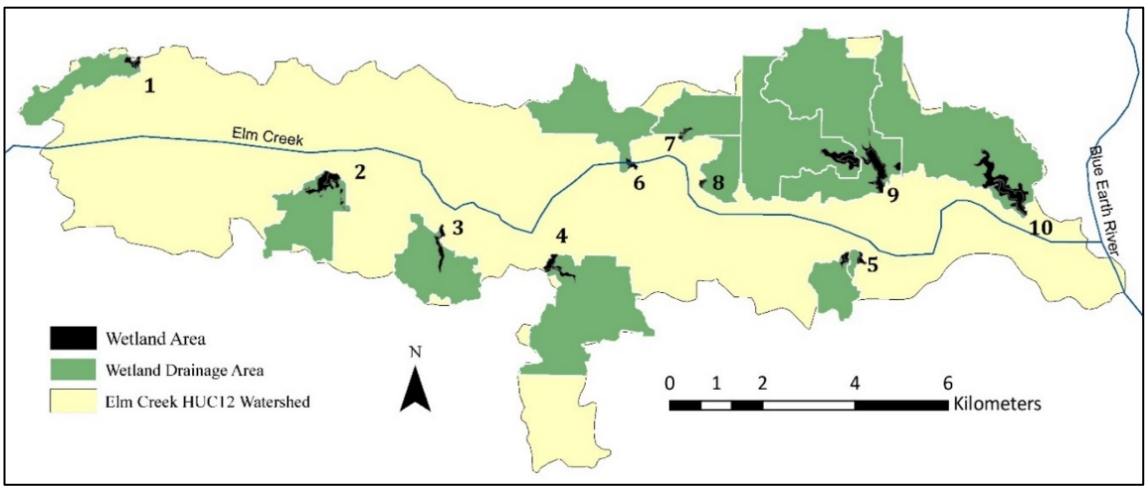

Figure 22. Large tile-drainage treatment wetland and watershed placements from the ACPF tool.

The cost effectiveness of the large wetlands ranged from \$1.31 kg^{-1} yr^{-1} to \$16.25 kg^{-1} yr^{-1} (mean = \$5.71 kg^{-1} yr^{-1}; standard deviation = \$5.80 kg^{-1} yr^{-1}). When all sites were combined, the cost effectiveness was \$3.18 kg^{-1} yr^{-1}. After comparing the cost effectiveness of the wetland sizes, the small, edge-of-field treatment wetlands were found to be significantly less cost effective when they had low conductivity compared to the small, high-conductivity wetlands or large wetlands ($p < 0.01$, Figure 23).

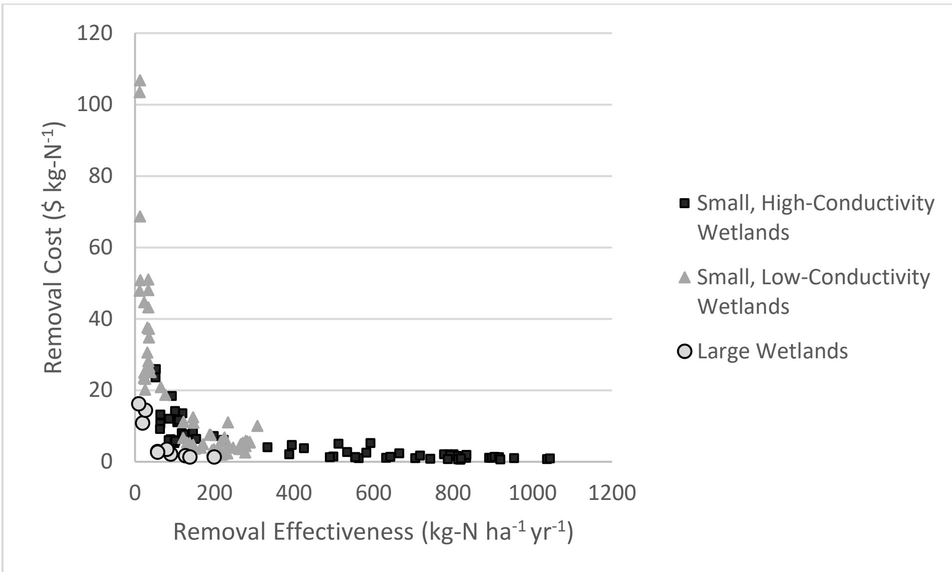

Figure 23. Comparison of nitrate removal and cost effectiveness of small, edge-of-field wetlands and large wetlands. The removal effectiveness is the mass removed for each hectare of wetland area.

Discussion

Although there is funding for treatment wetlands available through various sources such as the Farm Bill and Reinvest In Minnesota (Lucas 2014), these funds are limited. Funds are distributed each year, but nitrogen reductions are still falling short of the target in the states where a nutrient reduction strategy exists (IDALS 2016b; IEPA 2014; MPCA 2014). To make up the difference, investments in BMPs need to be in the most effective practices to allow the funds to pay for more reductions.

Currently, most wetlands designed to remove nitrogen require taking at least 2 ha of cropland out of production (Hyberg et al. 2015; IDA 2009). The ACPF tool uses a minimum watershed area of 60 ha for the nutrient removal wetland component, but Iowa requires a minimum of 200 ha to use CREP funding for wetlands. Nonetheless, wetlands in the ACPF tool follow the design suggestions and reduction potential from CREP wetlands in Iowa (Crumpton and Stenback 2016; Porter et al. 2016). These wetlands have been very successful at removing nitrate as more continue to be constructed throughout Iowa. However, there needs to be other options for tile drainage treatment for landowners who do not have neighbors willing to cooperate in constructing a large wetland, for landowners who are unwilling to retire large areas of their own cropland, or for farmers who need to maintain an annual income sufficient to support their operations. Furthermore, the fluctuating acreage of land in the conservation reserve program (CRP), and its potential impacts on CREP funding, is yet another reminder to seek practices that are the most economically effective (Lucas 2014). Therefore, if landowners are more likely to accept a practice that removes less land from production, it is important for agencies to know whether these more easily constructed practices are as effective or more effective by performance and cost. The models used in this study aided in comparing their effectiveness.

One difference between most of the locations from the ACPF tool and the Granada wetland is that the Granada wetland was in the historic floodplain, but most of the modeled sites were not. This could potentially lead to less infiltration due to non-alluvial soils – such as the glacial tills covering most of the uplands in the Elm Creek watershed – tend to have lower infiltration rates (Matzdorf 1989). However, the STATSGO soil database has very similar soils throughout the Elm Creek watershed which should lead to similar infiltration rates in each wetland depending on the compaction during construction. These similar infiltration losses should lead to similar subsurface reductions to those in the Granada wetland. However, wetlands with limited infiltration were also modeled in case water could not infiltrate in any given location.

One key finding in this study was that infiltration can be a valuable pathway for more nitrate removal in small wetlands. Allowing water to continue to receive treatment in shallow groundwater flow with long residence times can significantly improve the effectiveness in contrast to low denitrification rates in free water surface wetlands with flow rates that are too high. Having shallow groundwater treatment after water infiltrates from the surface of the wetland creates a dual-treatment system with a wetland on the surface and a saturated buffer-like system next to or under it (Jaynes and Isenhart 2014). However, this may not be possible in all locations. If the water table is too low or a drinking well is too close, shallow groundwater reductions may not be possible. Small wetlands with a low saturated hydraulic conductivity in this study still removed more nitrate per area retired than large wetlands ($p = 0.06$). Because the small wetlands were more effective, less land would be taken out of production for small wetlands to reach any nitrogen reduction goals than taken out for large wetlands to reach the goals.

While reductions of nitrogen for each hectare of wetland constructed were more effective for small, edge-of-field wetlands than large wetlands, the small wetlands were not more cost effective. The low conductivity, edge-of-field wetlands cost more for each kilogram of nitrogen removed than larger wetlands or high conductivity, edge-of-field wetlands, but the costs of high-conductivity wetlands were comparable to larger wetlands. Therefore, installing small, edge-of-field wetlands with high conductivity could remove less land from crop production but cost the same as installing large wetlands to remove the same mass of nitrate (Figure 23).

Although these smaller wetlands are comparable to larger designs in the Elm Creek watershed, there needs to be more research in other regions. More research should also be completed in different soil types and infiltration rates. The Elm Creek watershed is part of the Des Moines lobe, but even portions of this lobe farther south have other soil types including loess deposits that would have different drainages. Over 80% of the Elm Creek watershed is farmed and drained due to the poorly drained clays and loams throughout (Matzdorf 1989; USDA NRCS 2017). These soils are good for constructing wetlands, but sandier soils in other landscape settings may not benefit as greatly from the installation of nutrient treatment wetlands due to greater infiltration rates and more difficulty sealing the bottoms of wetlands with local soils.

Furthermore, the benefit of constructing smaller wetlands with higher conductivity is that the cost is comparable to larger wetlands, but less cropland needs to be taken out of production. This is not considering other benefits or ecosystem services of restoring wetlands. This study considers only nitrate removal. Future studies need to look at the wildlife habitat, floodwater storage, plant community, and recreation benefits of small wetlands versus large wetlands that are constructed primarily for tile drainage nitrate removal.

Conclusion

The first hypothesis was rejected due to the smaller wetlands being more effective at removing nitrogen for each area removed from crop production with $p = 0.06$ for low infiltration edge-of-field wetlands compared to large wetlands and $p < 0.001$ for high infiltration edge-of-field wetlands compared to the large wetlands. Although large wetlands remove large masses of nitrate, multiple small wetlands can play a significant role in removing nitrate loads from agricultural watersheds. This may be appealing to farmers because they can take fewer acres from production but remove just as much nitrate from tile drainage if they construct multiple small wetlands instead of one large wetland. The second hypothesis was confirmed due to smaller wetlands being similarly cost effective to the large wetlands except when the small wetlands had a low saturated hydraulic conductivity. The low conductivity, edge-of-field wetlands were significantly less cost effective than the large wetlands ($p = 0.003$), but the high conductivity, edge-of-field wetlands were not significantly different than the large wetlands. This is valuable information for state and federal agricultural agencies when deciding how to prioritize funds.

The study was conducted in a watershed dominated by glacial till soil with a high water table and high residence time for shallow groundwater nitrate removal, so the results are not applicable to watersheds with highly permeable soils. However, this study suggests that there is great value in investing in smaller wetland designs, especially if there is a dual treatment of surface water and shallow groundwater in each wetland. If funds are invested in small, edge-of-field wetlands instead of large wetlands, comparable masses of nitrate could be reduced while leaving more land in agricultural production. However, this should be studied in other watersheds with other soil types, and other ecosystem services of wetlands were not addressed in this study such as floodwater retention and habitat restoration. These services should be studied in small, edge-of-field wetlands before concluding which wetland size is a better investment altogether.

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Appendix 1

Table 29. Total phosphorus reductions in constructed wetlands from a literature review (Smith 2014).

References	TP% Reduced
(Braskerud et al. 2005)	1
(Braskerud et al. 2005)	16
(Braskerud et al. 2005)	43
(Braskerud et al. 2005)	29
(Braskerud et al. 2005)	11
(Braskerud et al. 2005)	27
(Braskerud et al. 2005)	20
(Braskerud et al. 2005)	10
(Braskerud et al. 2005)	28
(Braskerud et al. 2005)	42
(Braskerud et al. 2005)	7
(Braskerud et al. 2005)	83
(Braskerud et al. 2005)	23
(Braskerud et al. 2005)	23
(Braskerud et al. 2005)	62
(Braskerud et al. 2005)	23
(Braskerud et al. 2005)	88
(Fisher and Acreman 2004)	58
(Fisher and Acreman 2004)	0
(Fisher and Acreman 2004)	0
(Higgins et al. 1993)	99.5
(Higgins et al. 1993)	77.5
(Vellidis et al. 2003)	66
(Chescheir, Skaggs, and Gilliam 1992)	81
(Jordan et al. 2003)	39
(Jordan et al. 2003)	-15
(Magner et al. 1995)	27
(Kovacic et al. 2000)	17
(Kovacic et al. 2000)	10
(Kovacic et al. 2000)	35
(Kovacic et al. 2000)	80
(Kovacic et al. 2000)	-13
(Kovacic et al. 2000)	38
(Kovacic et al. 2000)	14.6
(Kovacic et al. 2000)	-14.8
(Kovacic et al. 2000)	-54
(Miller et al. 2012)	54.6
(MPCA 2016b)	40

Table 30. List of plant species seeded into the wetland after construction. The wetland code refers to the wetness tolerance of each species. The low, medium, and high diversity mixes are indicated on the right.

Scientific name	Common name	Wetland code	Low	Medium	High
Andropogon gerardii	Big Bluestem	FAC		x	x
Bromus ciliatus	Fringed Brome	FACW			x
Calamagrostis canadensis	Blue Joint Grass	OBL			x
Elymus canadensis	Canada Wild Rye	FACU	x	x	x
Glyceria grandis	Reed Manna Grass	OBL		x	x
Glyceria striata	Fowl Manna Grass	OBL			x
Panicum virgatum	Switchgrass	FAC	x	x	x
Poa palustris	Fowl Bluegrass	FACW	x	x	x
Sorghastrum nutans	Indiangrass	FACU			x
Spartina pectinata	Prairie Cord Grass	FACW		x	x
Carex pellita	Broad-leaved Woolly Sedge	OBL		x	x
Carex stricta	Tussock Sedge	OBL			x
Carex vulpinoidea	Brown Fox Sedge	FACW	x	x	x
Scirpus atrovirens	Green Bulrush	OBL	x	x	x
Scirpus cyperinus	Woolgrass	OBL	x	x	x
Anemone canadensis	Canada Anemone	FACW			x
Asclepias incarnata	Swamp Milkweed	OBL	x	x	x
Symphyotrichum puniceus	Swamp Aster	OBL	x	x	x
Symphyotrichum umbellatus	Flat-topped Aster	FACW			x
Desmodium canadense	Showy Tick Trefoil	FACU	x	x	x
Eupatorium maculatum	Joe Pye Weed	OBL	x	x	x
Eupatorium perfoliatum	Boneset	OBL			x
Helenium autumnale	Sneezeweed	FACW		x	x
Helianthus grosseserratus	Sawtooth Sunflower	FACW	x	x	x
Liatris pycnostachya	Prairie Blazingstar	FAC		x	x
Lobelia siphilitica	Great Blue Lobelia	OBL			x
Mimulus ringens	Monkey Flower	OBL		x	x
Pycnanthemum virginianum	Mountain Mint	FACW			x
Verbena hastata	Blue Vervain	FACW	x	x	x
Vernonia fasciculata	Common Ironweed	FACW		x	x
Veronicastrum virginicum	Culver's Root	FAC			x
Zizia aurea	Golden Alexanders	FAC			x
Avena sativa	Oat (as cover crop)	NA	x	x	x

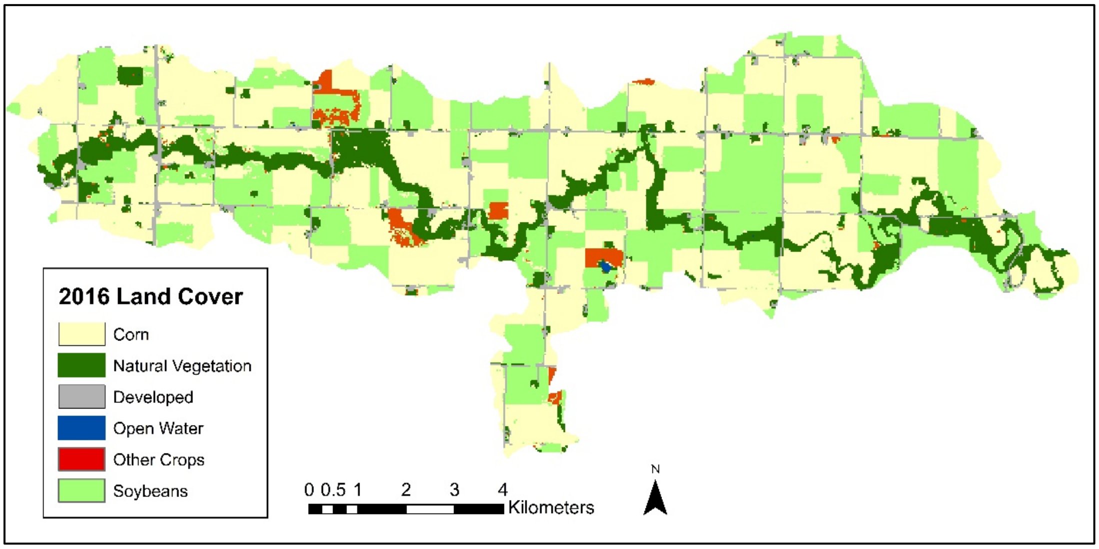

Figure 24. The HUC 12 Elm Creek watershed is comprised mostly of corn and soybeans (USDA 2017).

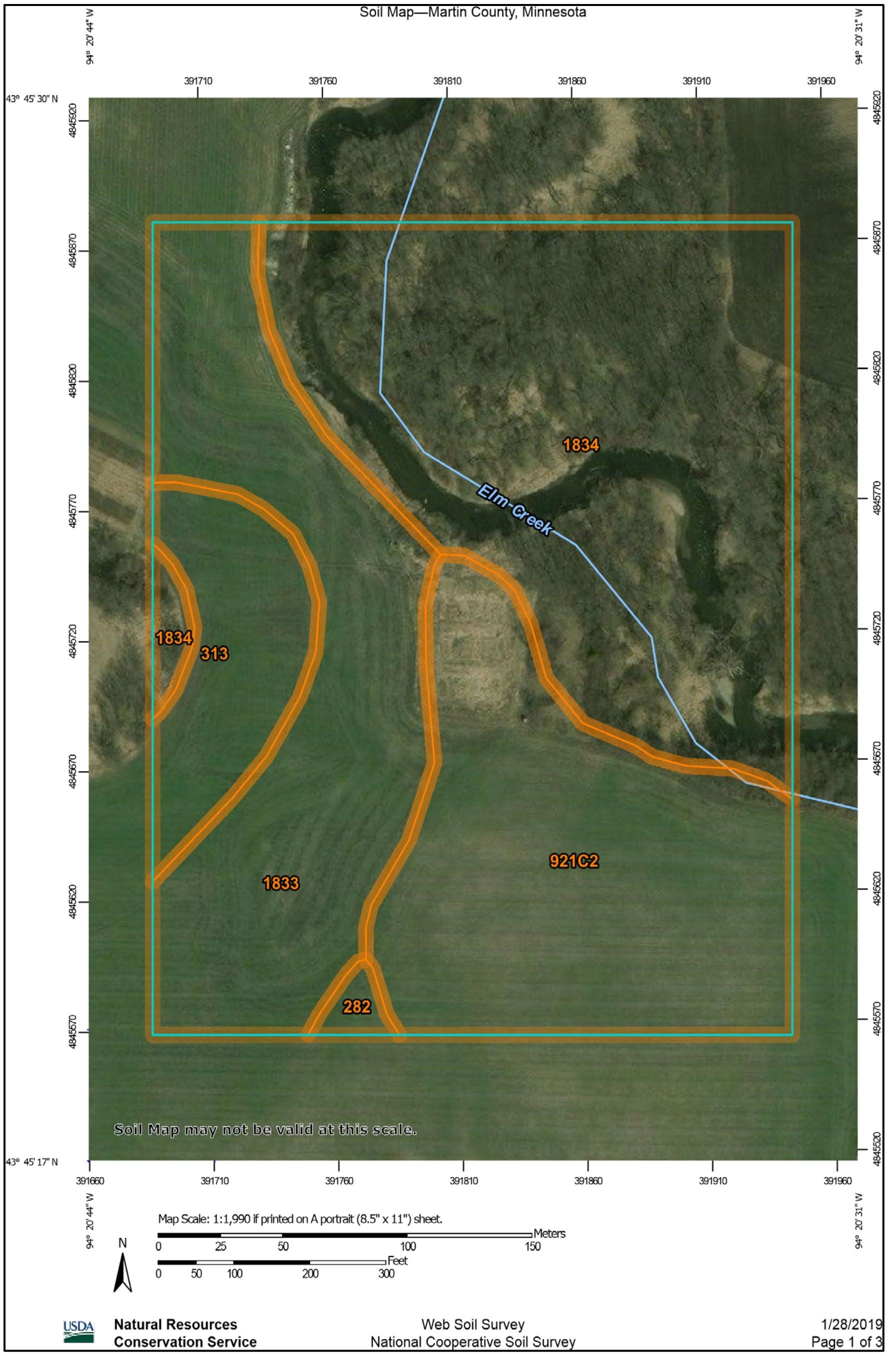

Figure 25. Soil map from USDA Soil Survey. The wetland soils include 921C2 – Clarion-Storden complex, 6-10% slopes, moderately eroded; 1833 – Coland loam, occasionally flooded; and 1834 – Coland loam, frequently flooded.

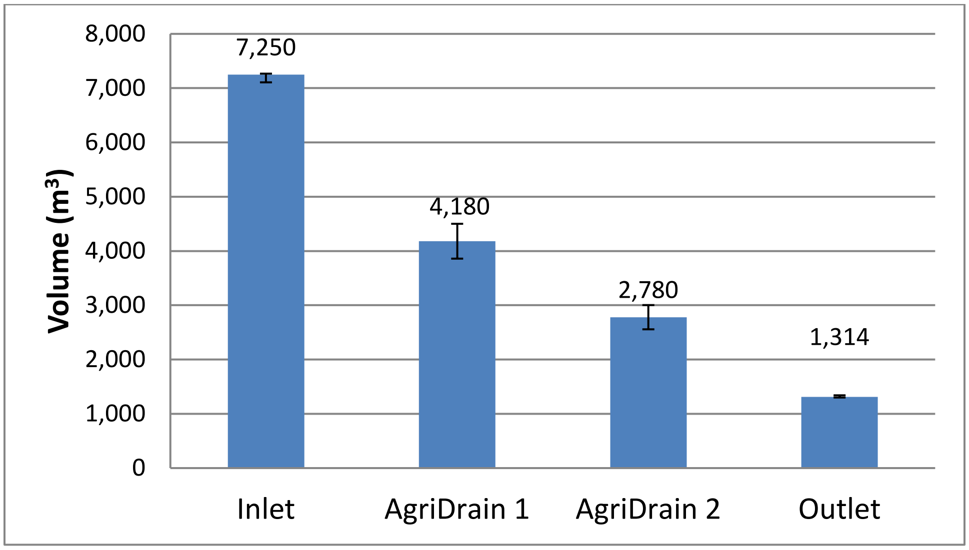

Figure 26. Water volumes flowing through the inlet, both Agri Drain structures, and the outlet in 2013. Error bars show estimated error calculated from instrument ranges and accuracies.

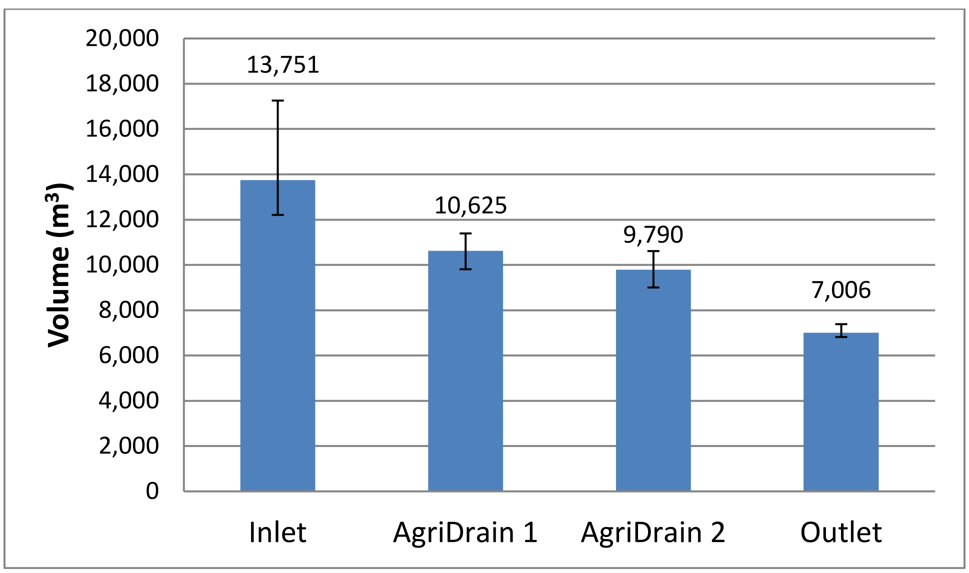

Figure 27. Water volumes flowing through the inlet, both Agri Drain structures, and the outlet in 2014. Error bars show estimated error calculated from instrument ranges and accuracies.

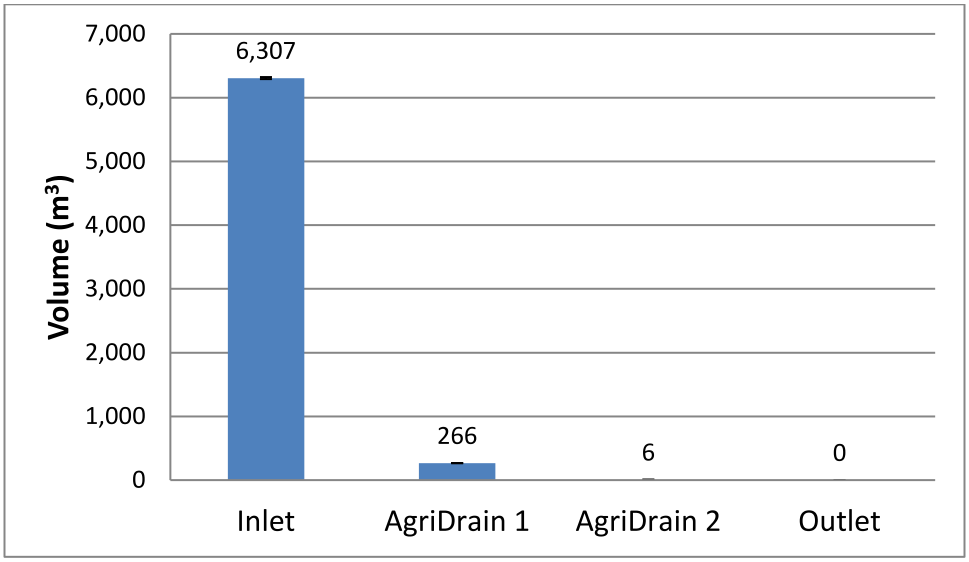

Figure 28. Water volumes flowing through the inlet, both Agri Drain structures, and the outlet in 2015. Error bars show estimated error calculated from instrument ranges and accuracies.

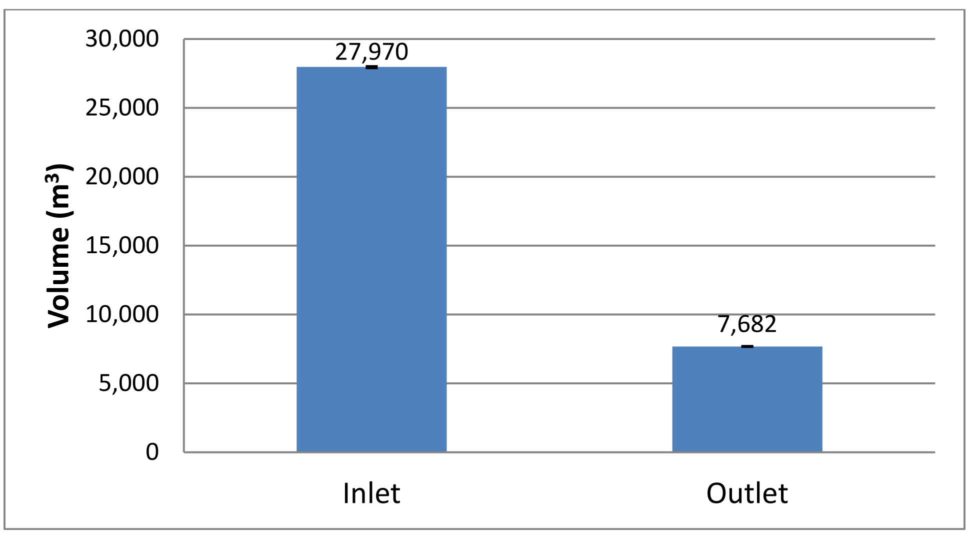

Figure 29. Water volumes flowing through the inlet and the outlet in 2016. Agri Drain 1 and 2 flow readings were unreliable due to flooding and slow flow throughout the field season. Error bars show estimated error calculated from instrument ranges and accuracies.

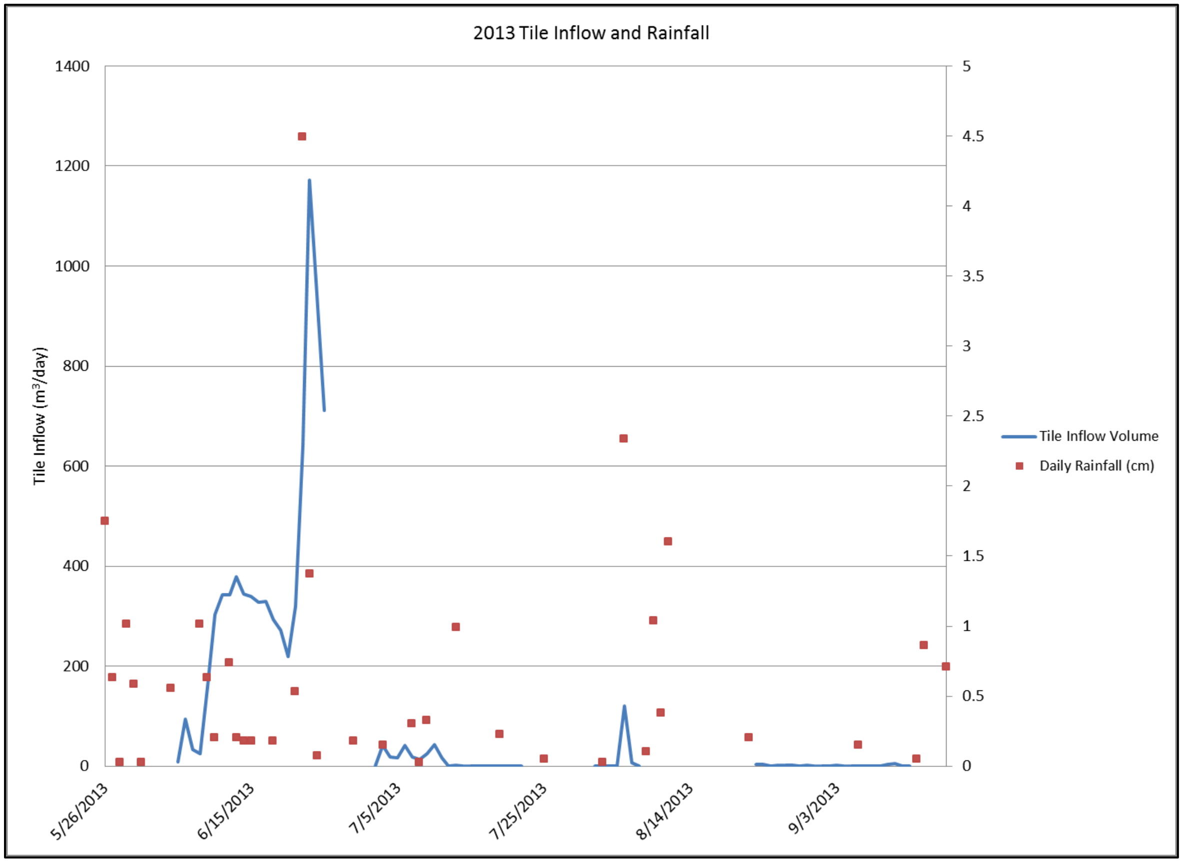

Figure 30. Hydrograph of the inlet tile paired with daily rainfall totals in 2013. The tile was connected to the wetland in early June, and the gaps in the graph represent flooding in late June and no flow later in the summer.

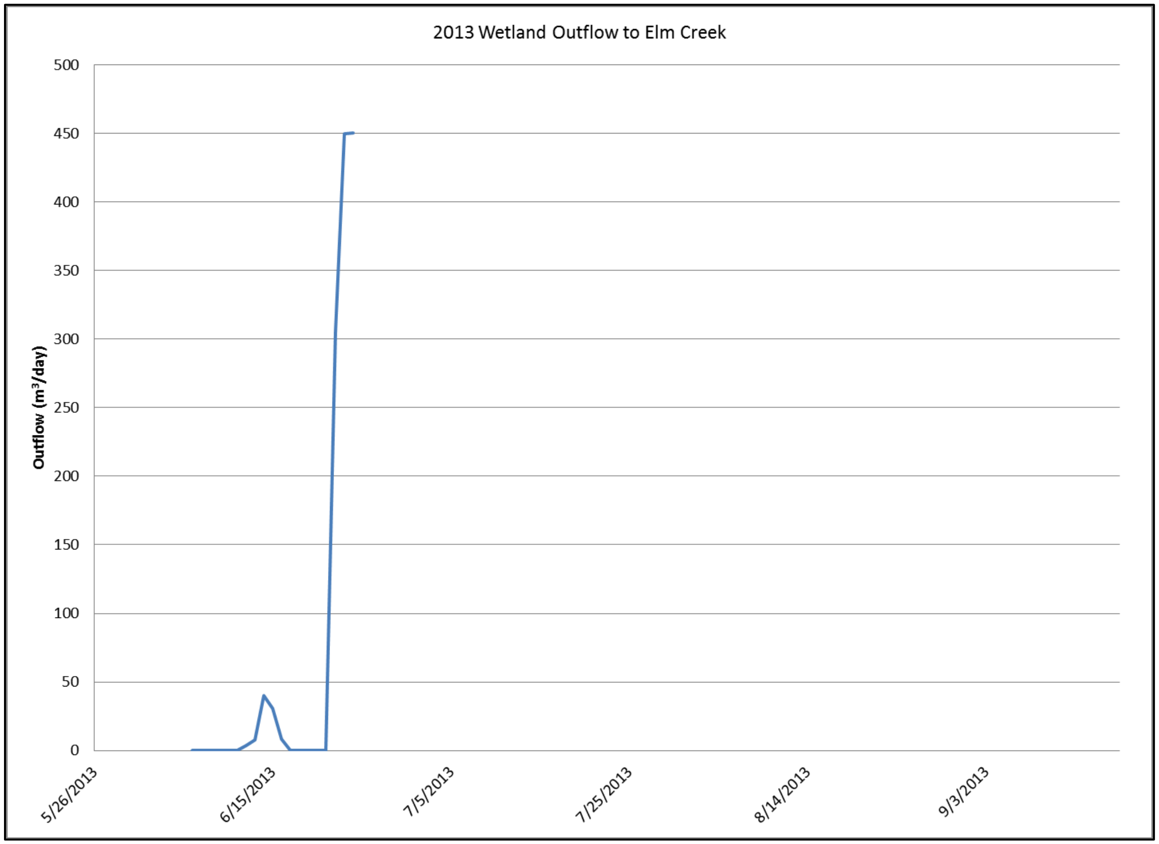

Figure 31. Hydrograph of the outlet discharge from the wetland to the creek in 2013. Flow began in early June. The graph ends in late June due to the flood and did not flow again the remainder of the summer.

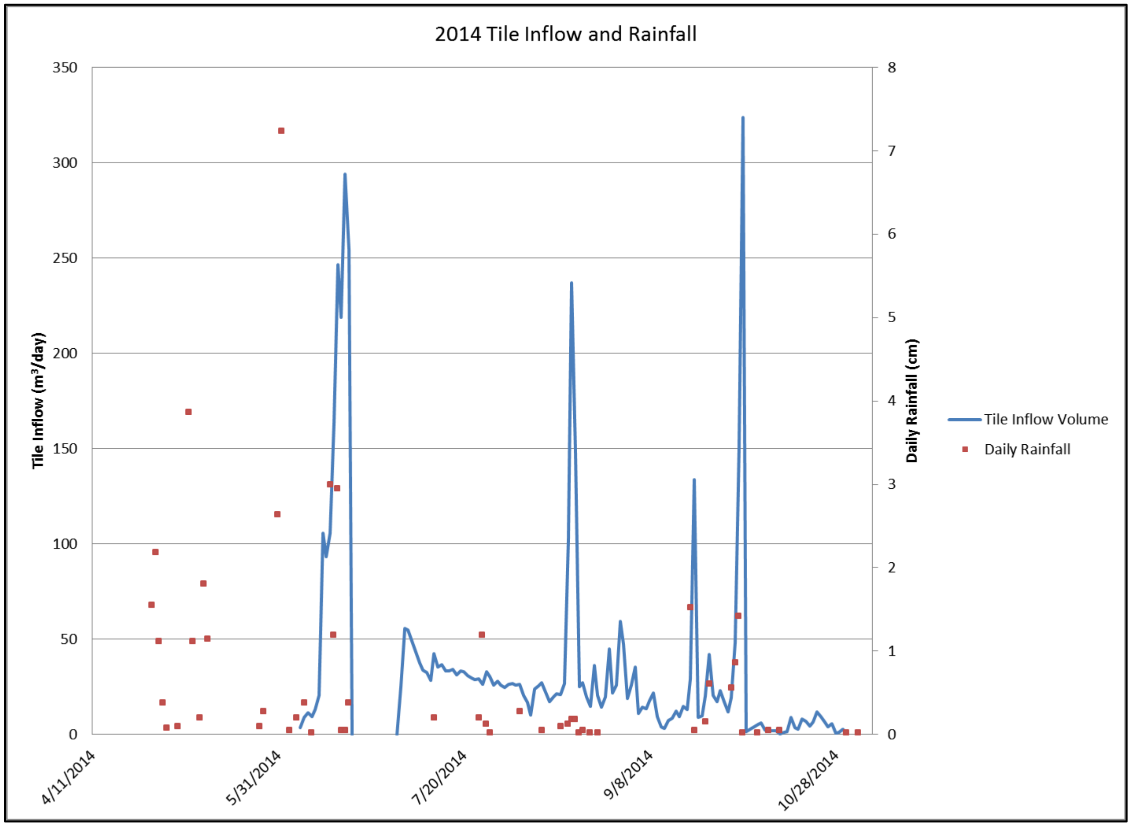

Figure 32. Hydrograph of the inlet tile paired with daily rainfall totals in 2014. The gap in the graph in late June is due to flooding. Inflow occurred before May, but it is not included in this graph due to the area velocity probe malfunctioning. Total volumes were estimated for that time using the Q:P ratio.

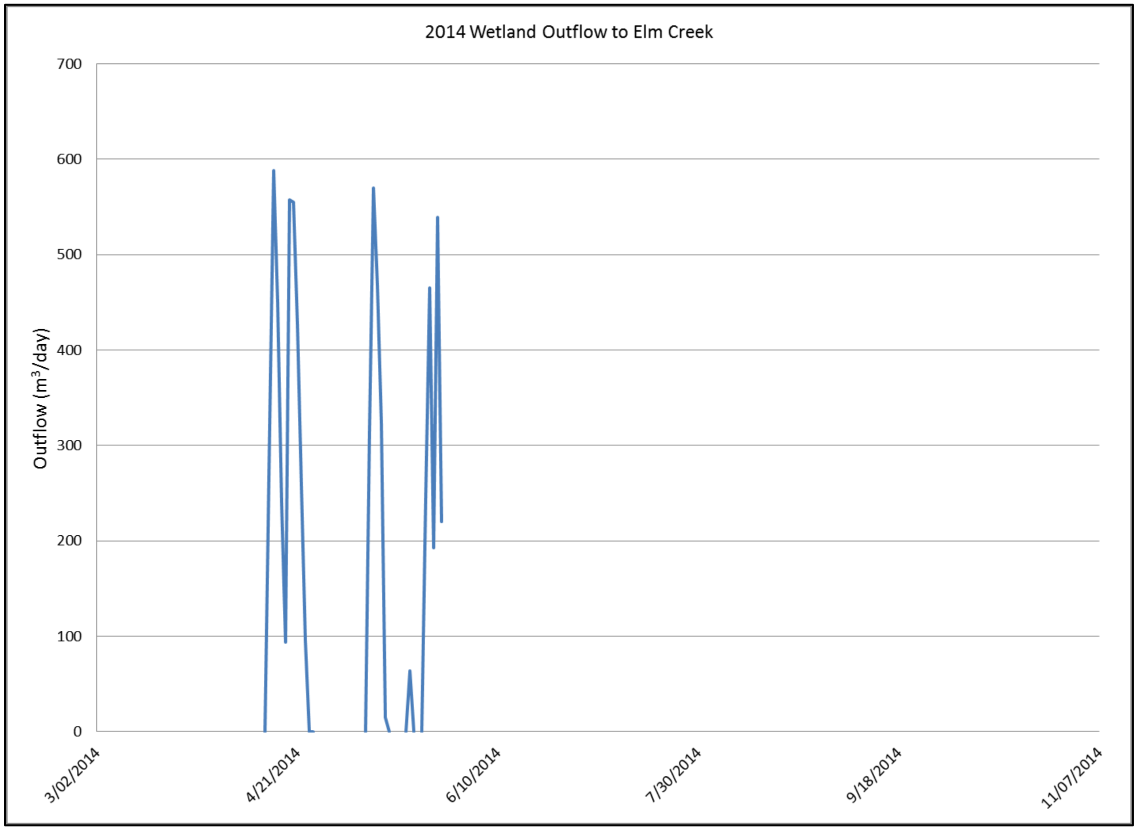

Figure 33. Hydrograph of the outlet discharge from the wetland to the creek in 2014. Discharge leading up to June 6th was estimated using the flow through Agri Drain 2 during that time due to the area velocity probe in the outlet malfunctioning. The graph ends in late June due to the flood and did not flow again the remainder of the summer.

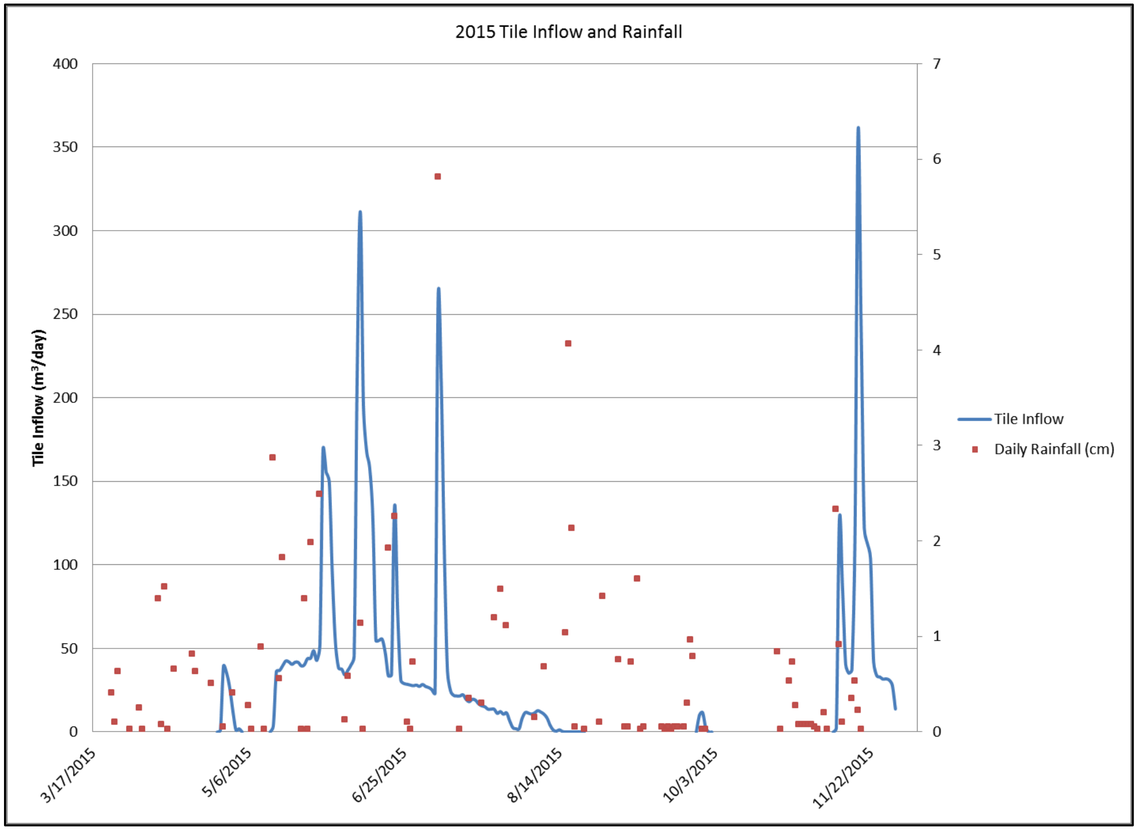

Figure 34. Hydrograph of the inlet tile paired with daily rainfall totals in 2015.

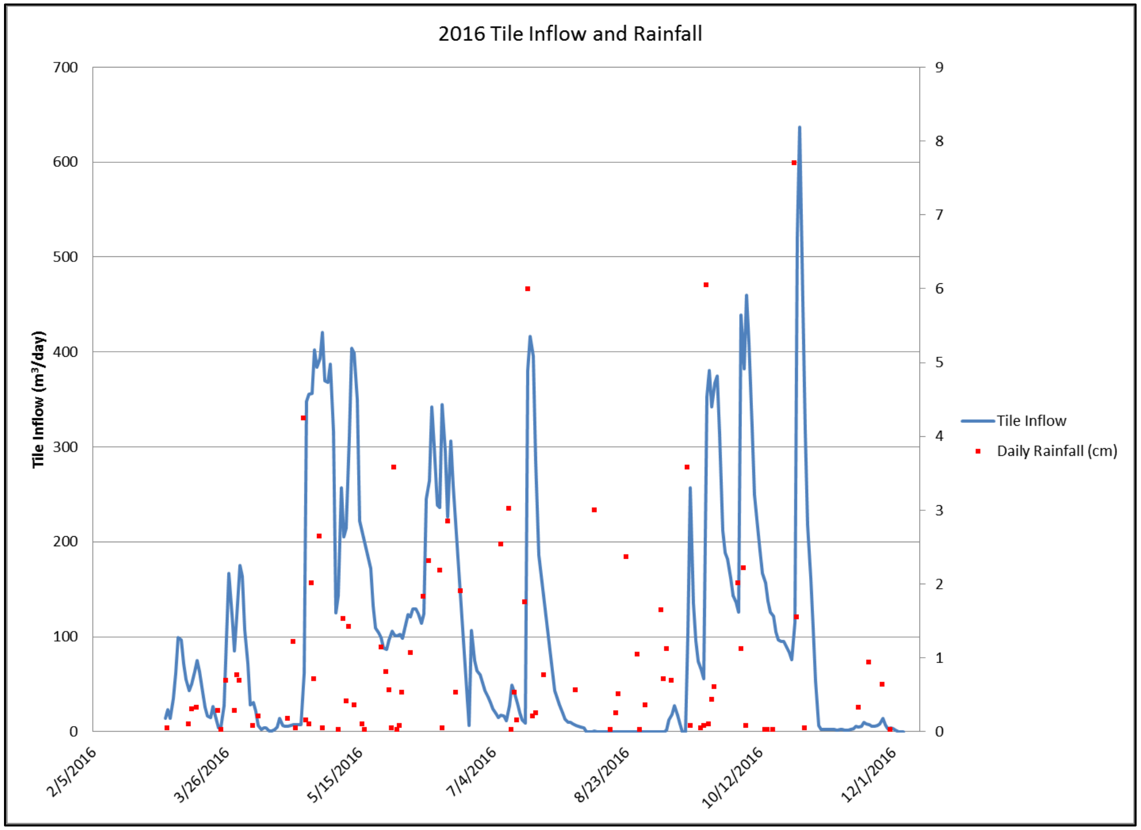

Figure 35. Hydrograph of the inlet tile paired with daily rainfall totals in 2016. Some short periods of flow were removed due to flooding, but they were not long enough in duration to be noticeable on this graph.

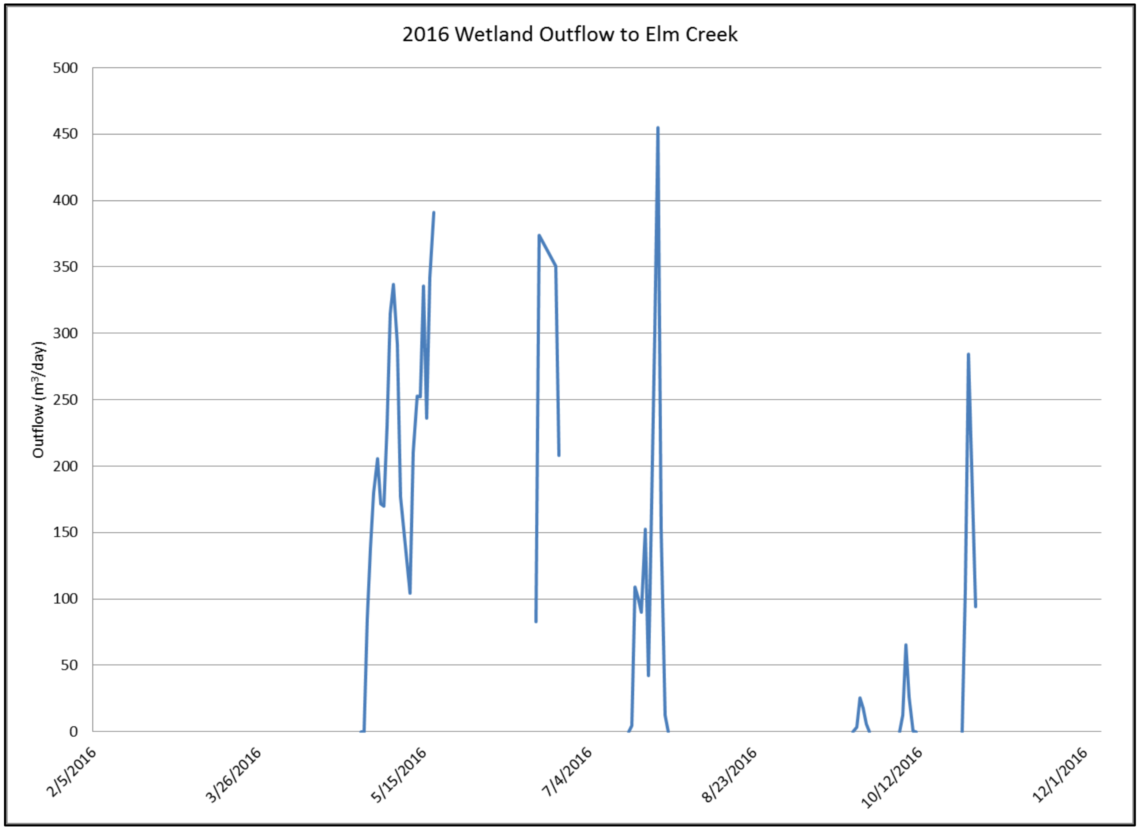

Figure 36. Hydrograph of the outlet discharge from the wetland to the creek in 2016. Gaps in the graph are due to multiple flooding events throughout the summer and no flow between late July and September.

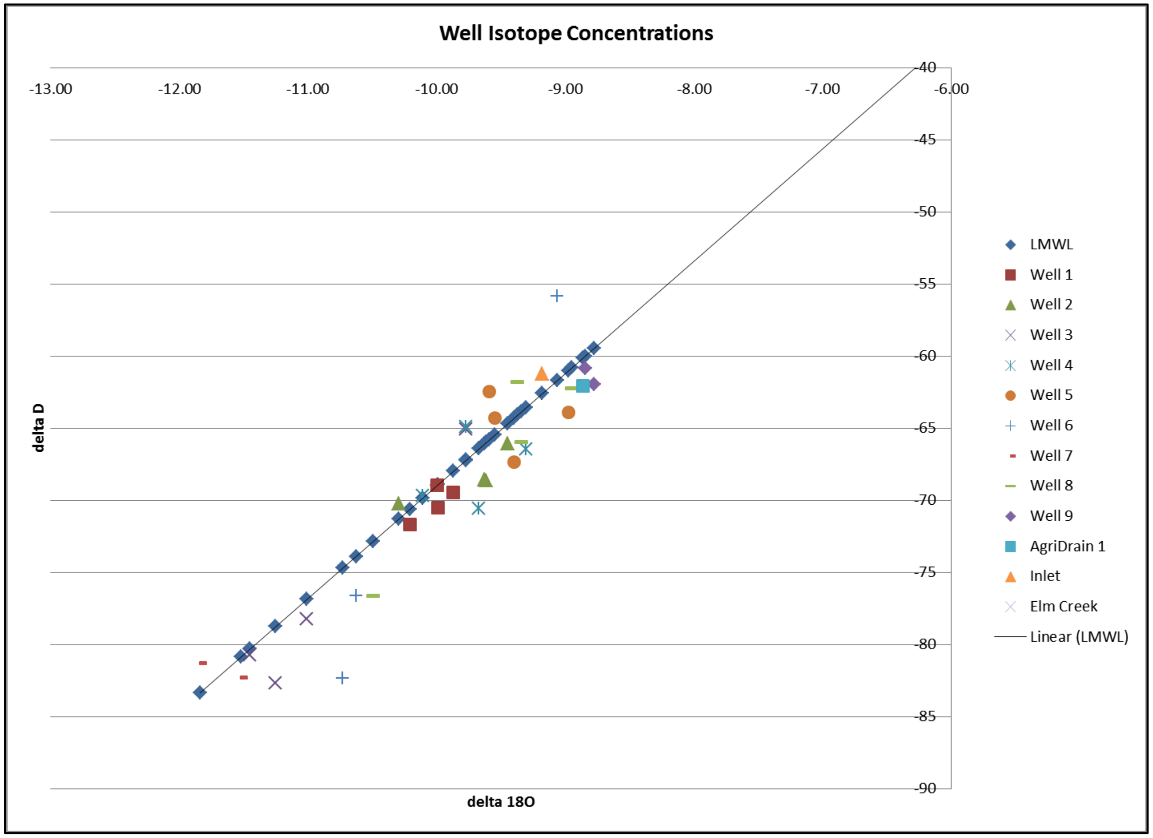

Figure 37. Isotope concentrations from wells and surface water at the wetland in 2015. Deuterium (δD) concentrations from Well 3, Well 5, Well 8, Inlet, and Agri Drain 1 were used to calculate the percent of water downgradient that came from groundwater versus surface water.

Table 31. Surface water residence time. These residence times are calculated from outflow rates from that cell.

	Median Cell 1 Residence Time (25^{th} – 75^{th} percentile, days)	**Median Cell 2 Residence Time (25^{th} – 75^{th} percentile, days)**	**Median Cell 3 Residence Time (25^{th} – 75^{th} percentile, days)**	**Median Wetland Residence Time (25^{th} – 75^{th} percentile, days)**
2013	0.64 (0.19-1.1)	0.050 (0.04-0.1)	3.4 (0.38-7.9)	10 (1.1-24)
2014	0.43 (0.25-0.92)	0.34 (0.18-0.63)	0.63 (0.33-1.2)	1.9 (0.98-3.7)
2015	2.2 (1.1 – 7.2)	NA	NA	NA
2016	0.17 (0.17-0.43)	0.16 (0.16-0.62)	0.88 (0.58-1.8)	2.6 (1.7-5.4)

Table 32. Volume and loads estimated using precipitation : drainage ratio in 2014.

Total Volume of Water (m^3)	**Nitrate/Nitrite-N Load (kg)**	**Total Phosphorus Load (kg)**	**Soluble Orthophosphorus Load (kg)**
8,761	145	1.14	0.961

Table 33. FLUX inflow estimates for all years combined. This includes the estimated flow during the missing data collection periods in 2014.

Total Days of Monitoring	Total Volume of Water (m^3)	Nitrate/Nitrite-N Load (c.v., kg)	Total Phosphorus Load (c.v., kg)	Soluble Orthophosphorus Load (c.v., kg)
812	56,050	826 (0.070)	5.98 (0.24)	4.54 (0.27)

Table 34. FLUX outflow estimates for all years combined. This includes the estimated flow during the missing data collection periods in 2014.

Total Days of Monitoring	Total Volume of Water (m^3)	Nitrate/Nitrite-N Load (c.v., kg)	Total Phosphorus Load (c.v., kg)	Soluble Orthophosphorus Load (c.v., kg)
756	17,910	237 (0.083)	2.45 (0.19)	1.34 (0.22)

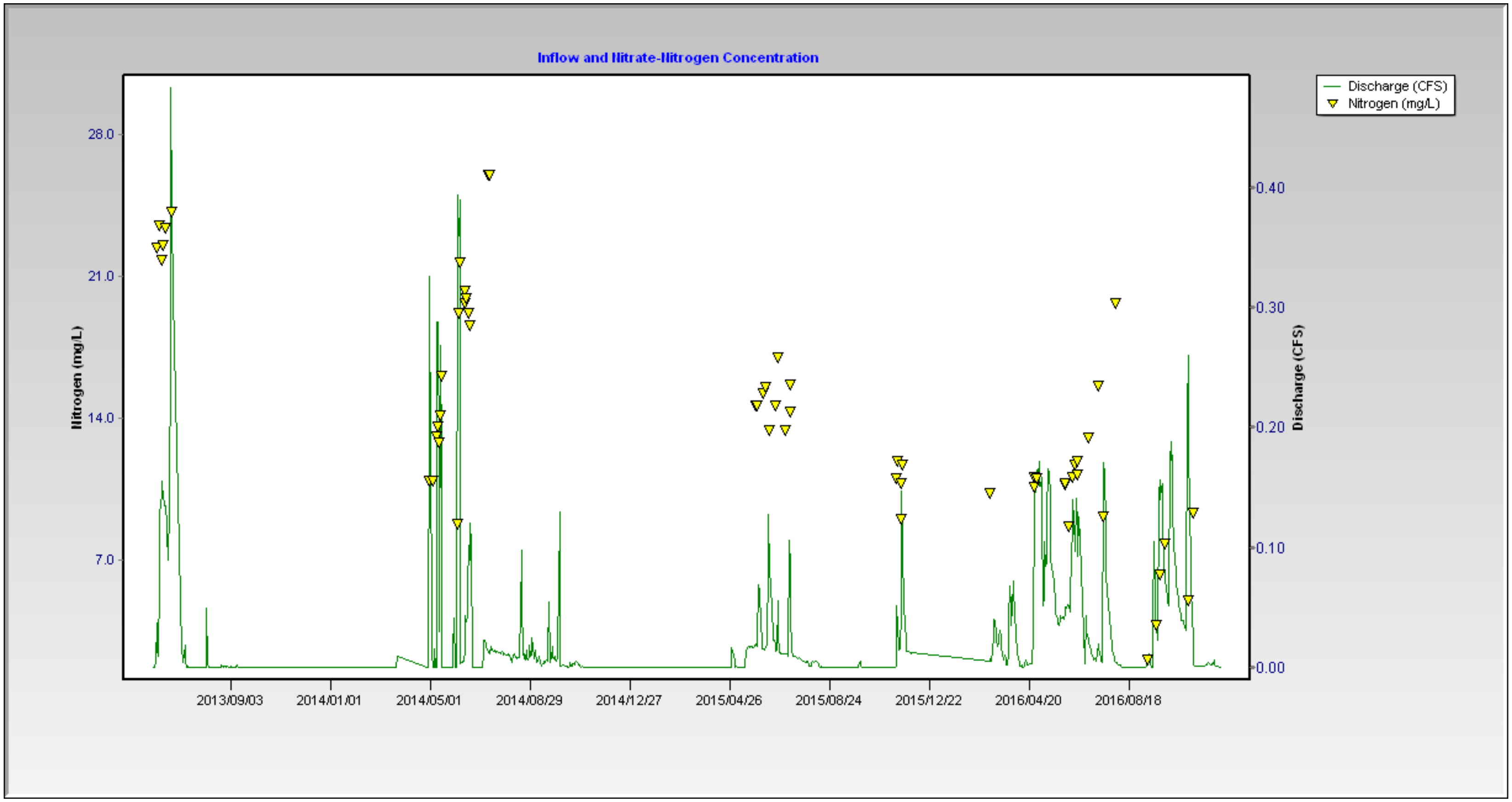

Figure 38. Inflow hydrograph and nitrate-N concentrations for all four years from FLUX.

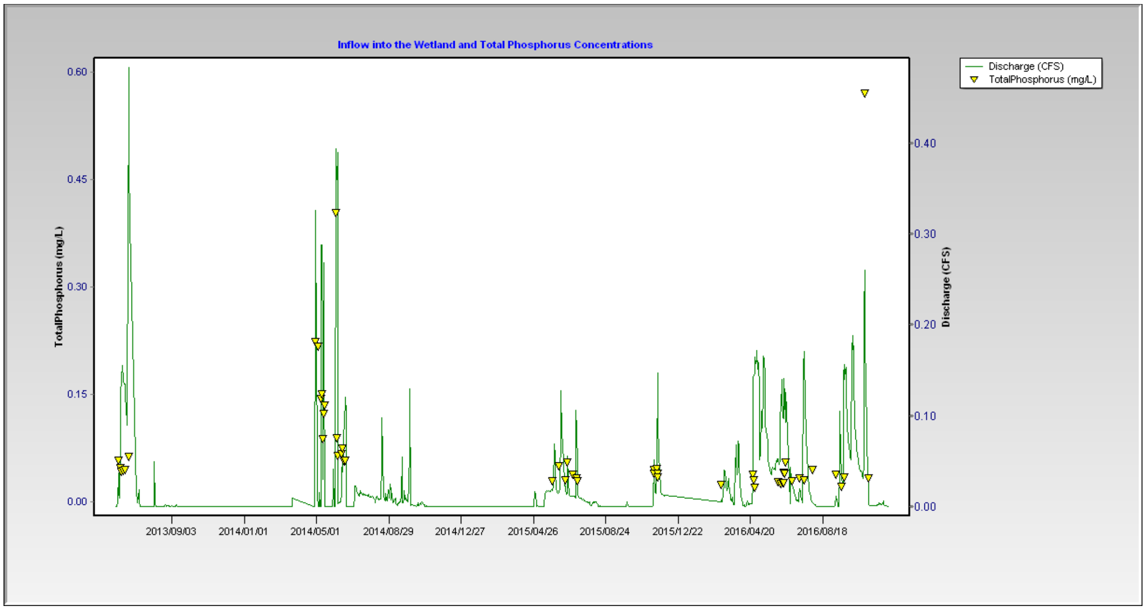

Figure 39. Inflow hydrograph and total phosphorus concentrations for all four years from FLUX.

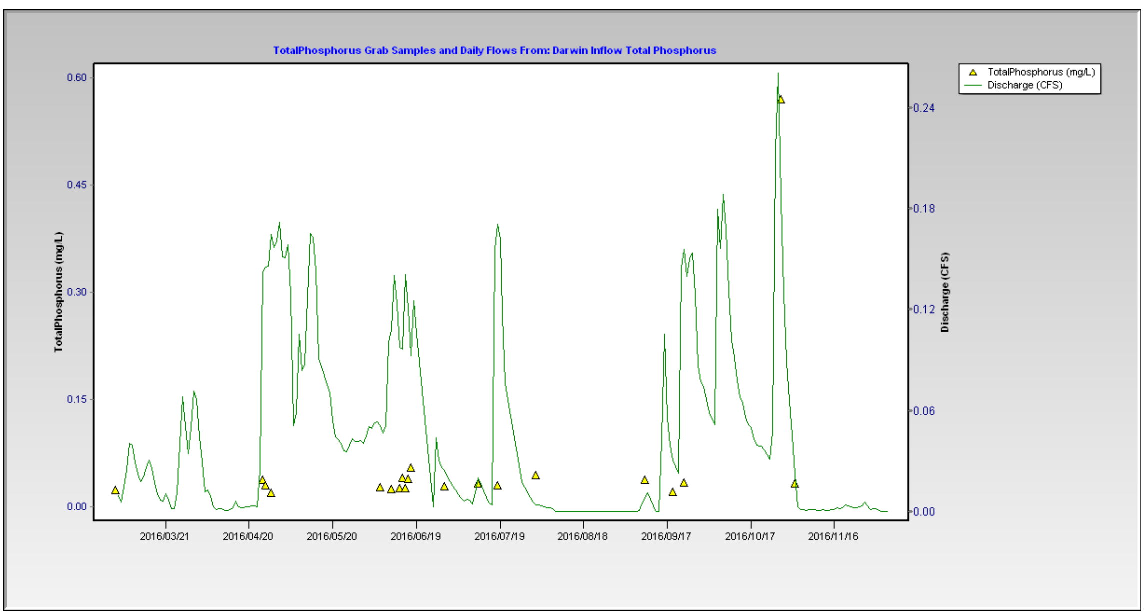

Figure 40. Inflow hydrograph and total phosphorus concentrations in the 2016 season from FLUX.

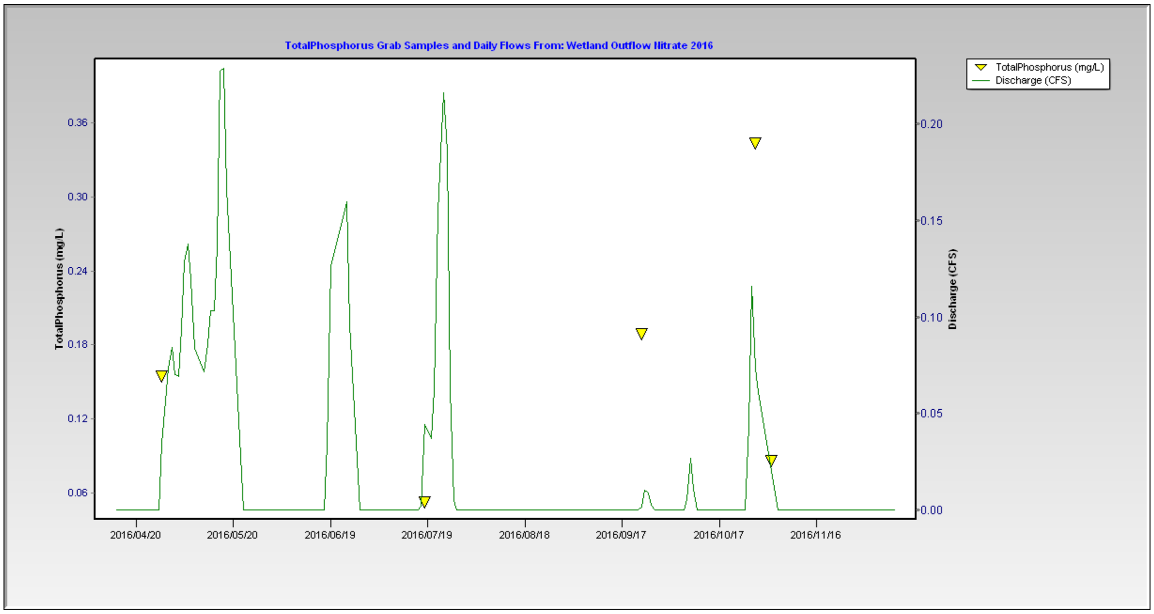

Figure 41. Outflow hydrograph and total phosphorus concentrations in the 2016 season.

Table 35. Summary of nitrate/nitrite-N loads each month in each of the three years.

	Nitrate/Nitrite-N Load by Month (kg)			
	2013	**2014**	**2015**	**2016**
March	0.0	0.300	0.0	16.1
April	0.0	21.0	1.50	26.8
May	0.0	57.3	14.7	56.0
June	178	80.9	36.1	41.8
July	3.60	15.2	16.3	21.0
August	3.30	18.2	1.20	0.500
September	0.300	9.90	0.300	34.1
October	0.0	9.80	0.100	66.7
November	0.0	0.0	17.8	2.80
December	0.0	0.0	0.0	0.100

Table 36. Summary of orthophosphorus loads each month in each of the three years.

	Orthophosphorus Load by Month (kg)			
	2013	**2014**	**2015**	**2016**
March	0.000	0.003	0.000	0.065
April	0.000	0.163	0.004	0.109
May	0.000	0.446	0.034	0.227
June	0.303	0.629	0.085	0.170
July	0.006	0.119	0.038	0.085
August	0.006	0.141	0.003	0.002
September	0.001	0.077	0.001	0.138
October	0.000	0.076	0.000	0.271
November	0.000	0.000	0.053	0.011
December	0.000	0.000	0.000	0.001

Table 37. Summary of total phosphorus loads each month in each of the three years.

	Total Phosphorus Load by Month (kg)			
	2013	**2014**	**2015**	**2016**
March	0.000	0.003	0.000	0.147
April	0.000	0.207	0.004	0.245
May	0.000	0.564	0.039	0.512
June	0.400	0.796	0.094	0.382
July	0.008	0.150	0.043	0.192
August	0.007	0.179	0.004	0.004
September	0.001	0.097	0.001	0.312
October	0.000	0.097	0.000	0.610
November	0.000	0.000	0.066	0.025
December	0.000	0.000	0.00	0.001

Appendix 2

Vegetation Percent Ground Cover in Mesocosms

Methods: After the experiment was complete, pictures were taken of each mesocosm. The photos were used to measure percent ground cover of the plants in each tank. Each photo was opened in Photoshop® and trimmed so the outline of the tank was the edge of the photo. The images were then converted to black and white after removing greens and blues to enhance the contrast between the plants and soil surface. Following this color conversion, the images were reduced to two to four degrees of pixel density. The degrees of pixel density varied by photo and depended on how well the plants were distinguished from the soil in each number of pixel densities. The histogram in Photoshop® was then used to read the percent of higher density pixels were in the photo. These higher density pixels represented the percent ground cover of the plants in each mesocosm. The results of these cover measurements were then used to make sure plant cover did not differ between tanks and therefore volatilization would not have differed between plant communities.

Results: Vegetation in each mesocosm grew quickly. Flowering amongst all the forbs and the switchgrass began in the middle of June and ended in late July. There was little to no flowering in the reed canary grass tanks. By the time the experiments began in November, vegetation in each tank was partially senesced. Reed canary grass was the least senesced of any of the plant communities. By the end of the experiment, the reed canary grass mesocosms had the greatest total plant growth of any of the plant communities with the greatest biomass both belowground and aboveground.

Percent ground cover in the mesocosms ranged from 33 to 72%. The average covers of the reed canary grass, switchgrass, and wet prairie mix tanks were 58%, 53%, and 41% respectively. However, the differences among the tanks' coverages were not significantly different. There was also no significant correlation between percent coverage and the mass of nitrogen removed during the experiment.

Discussion: A possible explanation for the differences in nitrate reduction among the plant communities includes the variability in volatilization of nitrous oxide and nitrogen gas among the mesocosms. This was an argument raised at a science conference where the results of this project were presented. The volatilization of these gases is the final step in the denitrification process. After denitrifying microbes convert nitrate to one of these gases, it still needs to escape from the water into the air above. This step can depend on vegetative cover and wind. In the lab, the mesocosms were not exposed to wind except for short periods when the doors to the lab were opened. These moments did not last more than minutes, so wind was not likely to differ among the mesocosms in this experiment. Ground cover or exposed water surface area may have differed among the mesocosms due to the variable growth of each plant community. However, the lack of a significant difference in ground cover among the plant communities indicates that variables in the volatilization process likely did not differ among the plant communities.

Therefore, removal of nitrate would not have been impacted by the rate of volatilization in this experiment.

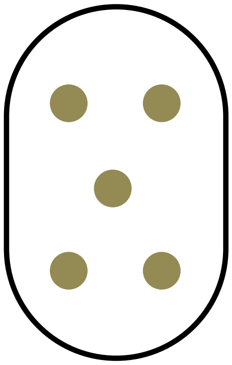

Figure 42. Below-ground biomass core collection locations in each mesocosm tank.

Table 38. Dry weight biomass of each mesocosm. Belowground biomass was estimated by extrapolating the masses of dry plant tissue in five soil cores from each mesocosm tank. All aboveground biomass from each tank was harvested, dried, and weighed.

Mesocosm	Below-Ground Biomass (g)	Above-Ground Biomass (g)	Estimated Below-Ground Biomass in Transplants (g)	Total Biomass Growth Since Planting (g)
Reed Canary 1	314.87	114.85	50.15	379.56
Reed Canary 2	442.37	211.46	65.78	588.05
Reed Canary 3	446.94	301.60	71.52	677.02
Switchgrass 1	93.23	49.56	15.93	126.85
Switchgrass 2	662.64	83.58	103.90	642.32
Switchgrass 3	106.02	79.61	26.84	158.80
Wet Prairie 1	530.11	206.30	58.60	677.81
Wet Prairie 2	453.34	234.93	61.96	626.30
Wet Prairie 3	64.89	128.92	14.43	179.39

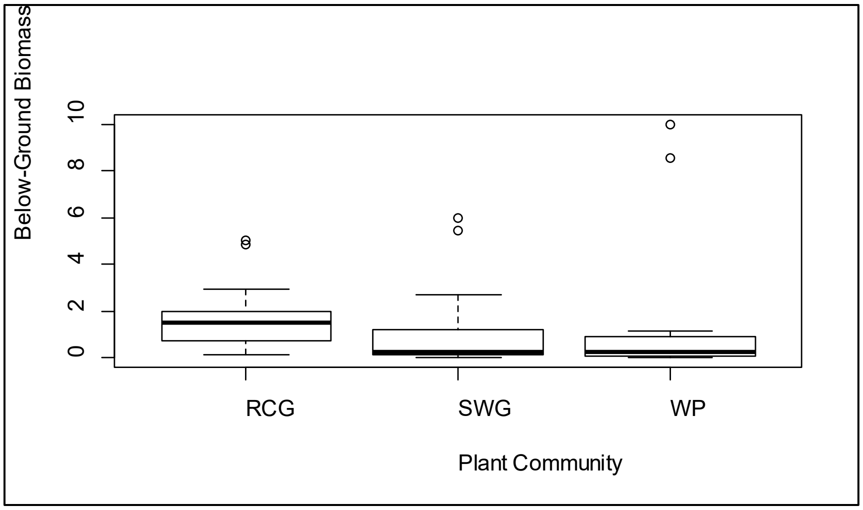

Figure 43. Dry mass of roots in each soil core in each plant community.

Table 39. Nitrogen and carbon mass composition measured through combustion analysis of plant tissue samples in each mesocosm.

Mesocosm Label	**Nitrogen in Below-Ground Tissue (%)**	**Nitrogen in Above-Ground Tissue (%)**	**Carbon in Below-Ground Tissue (%)**	**Carbon in Above-Ground Tissue (%)**
Reed Canary 1	1.3	2.1	43.6	41.9
Reed Canary 2	1.4	2.0	41.6	40.7
Reed Canary 3	1.2	2.2	43.1	41.2
Switchgrass 1	1.3	1.3	39.0	40.2
Switchgrass 2	1.1	1.1	32.8	44.5
Switchgrass 3	0.9	1.5	43.6	43.2
Wet Prairie 1	2.0	1.4	43.8	39.1
Wet Prairie 2	6.4*	1.3	41.6	43.2
Wet Prairie 3	2.4	1.3	39.0	40.8

*Wet Prairie 5's below-ground nitrogen measurement exceeded the maximum value in the standard curve. Therefore, the value above is the maximum standard value rather than the measured value.

Table 40. Estimated mass of nitrogen (N) and carbon (C) in the plant tissue of each mesocosm.

Mesocosm Label	Above-Ground N (g)	Below-Ground N (g)	Total N (g)	Above-Ground C (g)	Below-Ground C (g)	Total C (g)	Above-Ground C/N ratio	Below-Ground C/N ratio
Reed Canary 1	2.35	4.12	6.48	48.12	137.16	185.28	20.44	33.25
Reed Canary 2	4.21	6.10	10.31	86.06	183.94	270.00	20.45	30.13
Reed Canary 3	6.76	5.41	12.16	124.11	192.45	316.56	18.37	35.59
Switchgrass 1	0.64	1.20	1.84	19.92	36.33	56.25	30.92	30.21
Switchgrass 2	0.89	7.16	8.05	37.20	217.28	254.48	41.60	30.36
Switchgrass 3	1.19	0.99	2.18	34.35	46.17	80.52	28.77	46.83
Wet Prairie 1	2.79	10.55	13.33	80.62	231.98	312.60	28.95	21.99
Wet Prairie 2	3.08	29.01	32.09	101.44	188.63	290.08	32.96	6.50
Wet Prairie 3	1.68	1.52	3.20	52.56	25.29	77.85	31.36	16.58

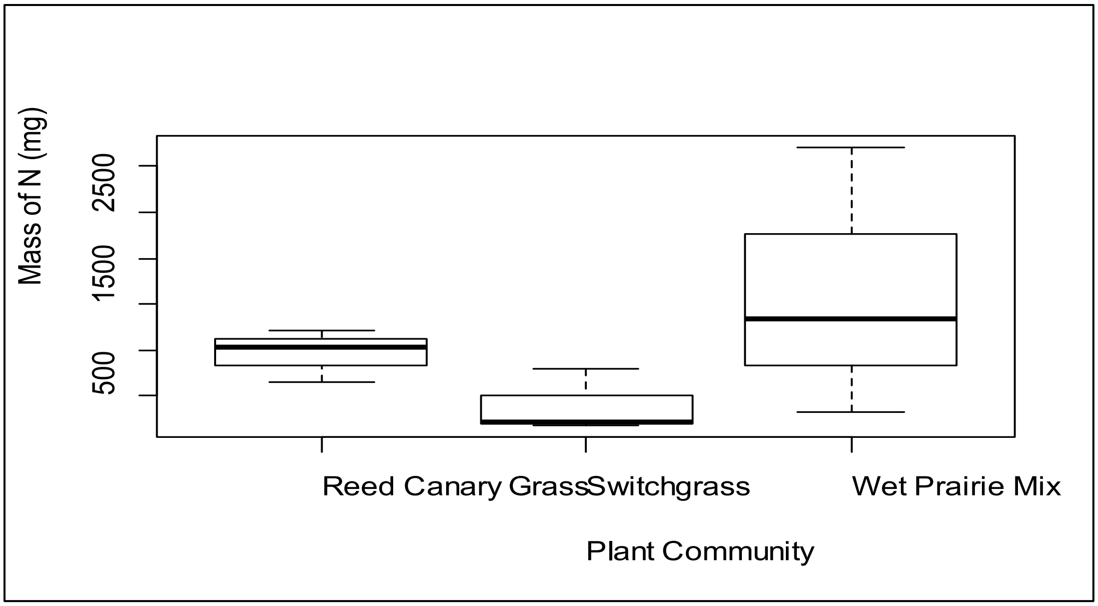

Figure 44. Total mass of nitrogen assimilated in plant tissue. There was no significant difference among the plant communities. One wet prairie mix sample was removed due to its exceedance of the instrument’s limits.

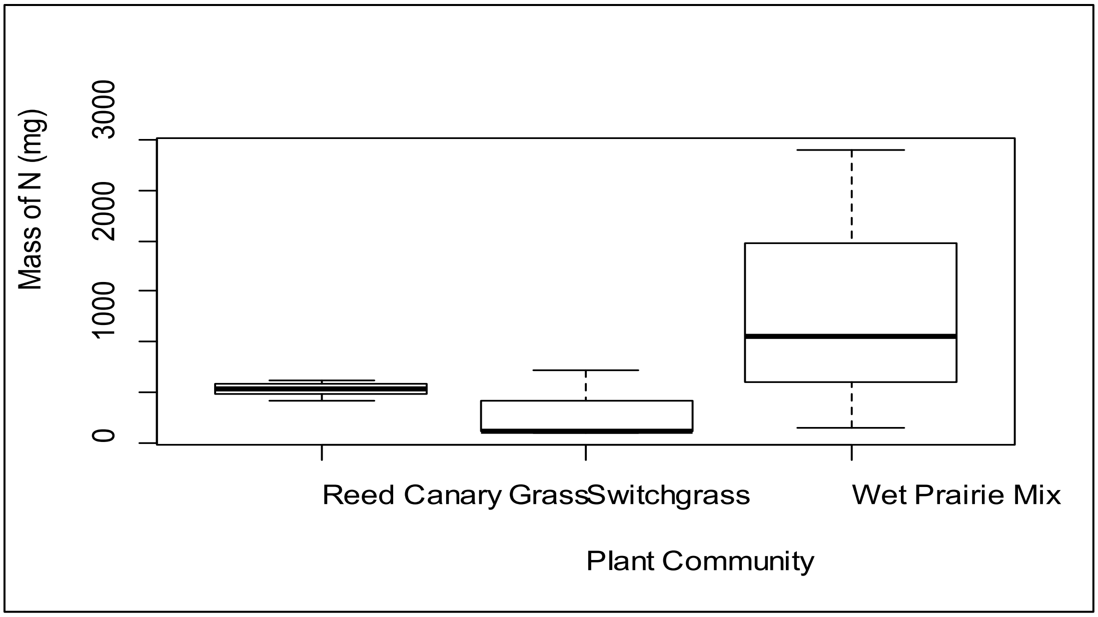

Figure 45. Below-ground mass of nitrogen assimilated in plant tissue. There was no significant difference among the plant communities. One wet prairie mix sample was removed due to its exceedance of the instrument's limits.

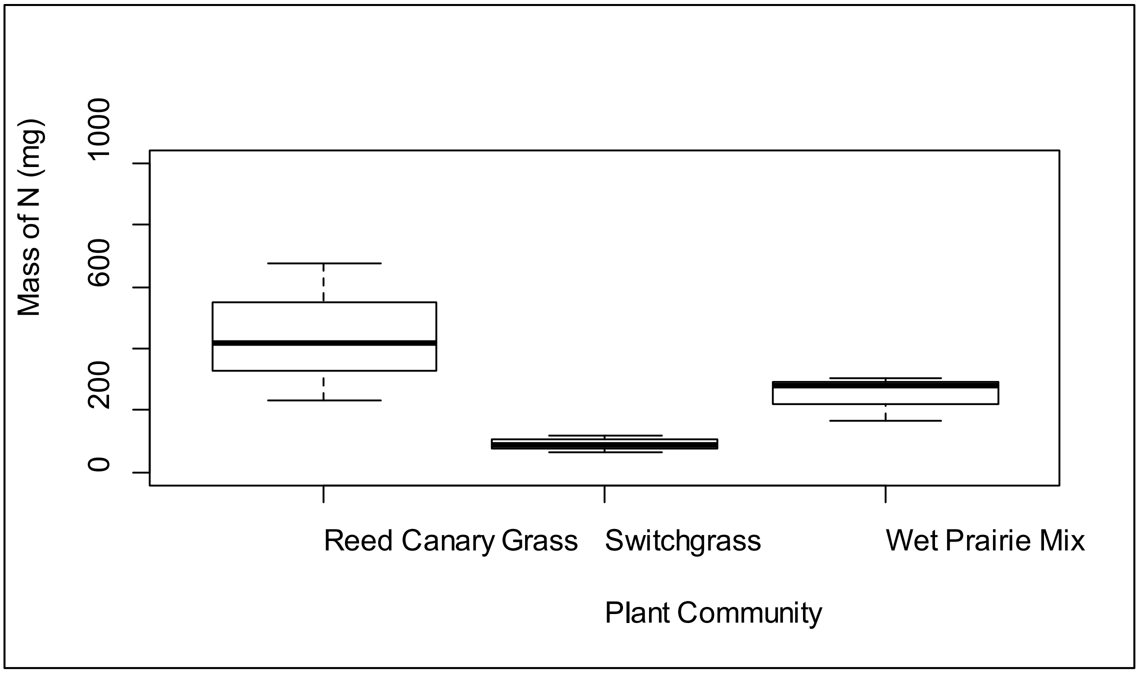

Figure 46. Above-ground mass of nitrogen assimilated in plant tissue. The switchgrass mix tanks were significantly lower in nitrogen mass than the reed canary grass tanks (p=0.04). Otherwise, there was no significant difference among the plant communities.

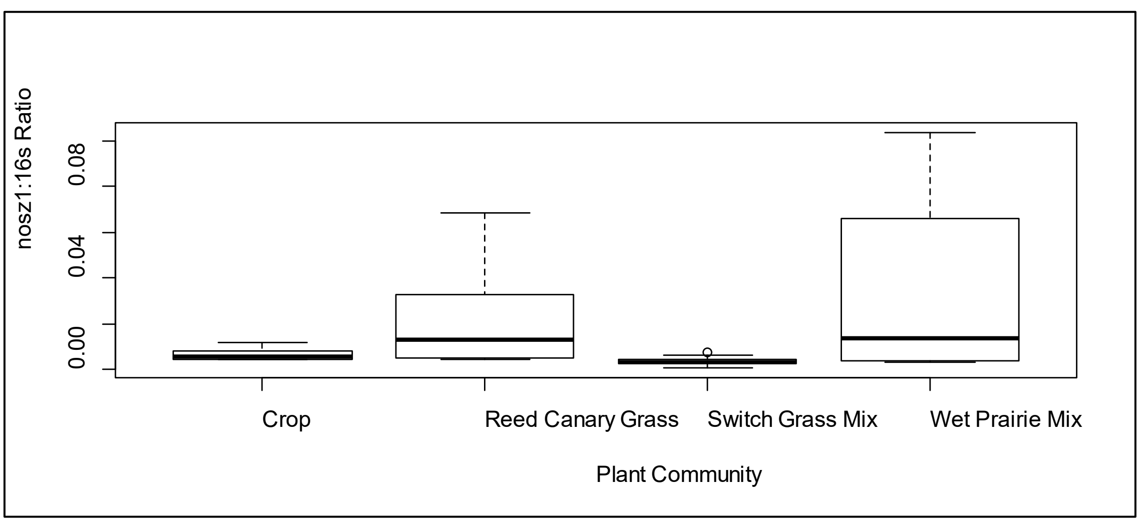

Figure 47. Ratio of *nos*Z1 to 16S copies from the row crop field and the three plant communities. There were no significant differences among the communities.

Appendix 3

When modeling locations for bioreactors in the Elm Creek watershed using the ACPF toolbox, a 1-meter DEM from the Minnesota DNR MnTOPO website was downloaded for the Elm Creek watershed (MnDNR 2017). Land use, field boundary polygons, and gSSURGO soil were downloaded from the ACPF Watershed Database Land Use Viewing and Data Downloading website (Porter et al. 2016). The tool determined where tile drainage was likely located in the watershed and then where bioreactors could ideally be constructed. For the tile drainage classification step of the model, Query 2 was selected {>= 90% of field is less than 5% slope ***OR*** >= 40% of field consists of a dual drainage hydrologic group (A/D, B/D, or C/D) or D class soil} for classifying fields where tile would be located. The ACPF tool then created a map of the Elm Creek watershed with a polygon output locating all the bioreactor locations and watershed sizes for each bioreactor.

Both STATSGO and SSURGO soil databases were used in the early SWAT calibration process until STATSGO was determined to yield closer results to the measured hydrology at the Roberts wetland. Three slope classes were developed including 0-1%, 1-2%, and 2+% slopes. Hydrologic Response Unit definitions were made with 20% land use, 20% soil class, and 5% slope class thresholds. Calibration and validation were performed by adjusting input parameters until modeled tile discharge was within 10% and had a Nash-Sutcliffe coefficient of efficiency (NSE) value of >60%. Nitrate load from tile drainage was calibrated and validated until the modeled load was within 10% and the NSE value was >60% (Arnold et al. 2012; Geza and McCray 2008;

Moriasi et al. 2007). Due to the limited number of years of measurements, along with 2013 and 2014's flow measurements being incomplete, calibration included 2015 and 2016, and validation included 2013, 2015, and 2016 together.

Table 41. Calibration parameters for the SWAT model.

Input Parameter	**SWAT Parameter**	**Default Value**	**Calibrated Value**
Depth to impervious layer (mm)	.hruDEP_IMP	0	1080
Depth to drain (mm)	.mgtDDRAIN	0	980
Time to drain to field capacity (hrs)	.mgtTDRAIN	0	72
Drain tile lag time (hrs)	.mgtGDRAIN	0	72
SCS runoff curve	.mgtCN2	~70	61
Available water capacity (mm H_2O/mm soil)	.solSOLAWC-layer 1	0.17-0.26	0.01
Denitrification threshold water content	.bsnSDNCO	1.1	0.57
Denitrification exponential rate coefficient	.bsnCDN	1.4	0.25
Nitrate percolation coefficient	.bsnNPERCO	0.2	1
Nitrogen uptake distribution parameter	.bsnN_UPDIS	20	100
Rate factor for humus mineralization	.bsnCMN	0.0003	0.003

Table 42. ACPF tool outputs for the small, edge-of-field treatment wetlands after confirming the watershed dimensions with SWAT.

Wetland Number	Drainage Area (ha)	Wetland + Buffer Area (ha)	Mass Discharged into Wetland (kg yr^{-1})
1	23.87	0.50	759
2	33.41	0.71	561
3	16.44	0.35	506
4	8.45	0.18	129
5	17.56	0.38	438
6	9.24	0.20	293
7	11.48	0.25	344
8	24.11	0.52	403
9	16.40	0.35	459
10	29.35	0.63	645
11	28.20	0.58	663
12	27.68	0.58	512
13	26.07	0.54	409
14	16.73	0.35	461
15	48.02	1.02	1,180
16	12.95	0.27	405
17	9.14	0.20	145
18	9.53	0.20	294
19	10.49	0.23	63.4
20	9.85	0.21	309
21	9.25	0.20	268
22	16.90	0.37	70.3
23	9.50	0.20	38.7
24	30.97	0.66	88.9
26	8.72	0.19	31.3
27	16.96	0.37	462
29	10.74	0.23	295
30	13.91	0.30	407
31	39.10	0.83	940
32	4.78	0.10	151
33	26.07	0.54	54.3
34	26.28	0.54	512
35	10.61	0.23	195
36	35.90	0.75	946
37	31.20	0.66	745

38	22.56	0.48	566
39	33.54	0.70	88.7
40	32.51	0.68	69.5
42	13.73	0.29	48.7
43	12.10	0.26	47.3
44	37.42	0.80	99.6
45	10.65	0.23	39.9
46	20.30	0.42	43.3
47	5.62	0.12	141
48	10.44	0.23	200
49	37.37	0.80	113
50	12.36	0.27	23.1
51	25.03	0.52	360
52	16.03	0.33	43.4
53	31.07	0.66	95.7
54	11.07	0.24	103
55	20.78	0.44	201
56	20.33	0.42	358
57	10.01	0.21	251
58	17.30	0.37	62.3
59	12.38	0.27	60.2
60	11.10	0.24	24.0
61	11.54	0.24	84.0
62	12.82	0.27	312
63	17.34	0.37	62.9
64	16.15	0.35	70.2
65	30.37	0.63	412
Total	1192	25.27	18,200
Mean	19.22	0.41	293
SD	9.95	0.21	262

Table 43. ACPF tool outputs for the large wetlands after confirming the watershed dimensions with SWAT.

Wetland Number	Drainage Area (ha)	Wetland + Buffer Area (ha)	Mass Discharged into Wetland (kg yr^{-1})
1	128.0	4.00	3,262
2	193.8	15.2	2,657
3	189.6	6.81	3,302
4	382.4	8.28	5,808
5	187.7	5.13	326.5
6	226.3	2.44	4,935
7	132.8	2.23	1,204
8	80.19	1.34	239.6
9	1204	36.7	5,816
10	523.4	36.5	488.5

Table 44. Outputs from the spreadsheet model of nitrate-N reductions in each small, edge-of-field wetland.

Wetland Number	Ksat = 0.00008 m day^{-1}			Ksat = 0.17 m day^{-1}		
	Mass Removed (kg)	% Mass Removed	Removal Efficiency (kg ha^{-1})	Mass Removed (kg)	% Mass Removed	Removal Efficiency (kg ha^{-1})
1	1389	18.3%	2773	5193	68.4%	10370
2	1069	19.1%	1506	3992	71.2%	5626
3	954.1	18.8%	2689	2332	46.1%	6572
4	256.2	19.8%	1461	610.8	47.2%	3484
5	874.0	19.9%	2327	2294	52.3%	6107
6	577.3	19.7%	2881	1303	44.6%	6504
7	665.8	19.3%	2659	1460	42.4%	5829
8	716.9	17.8%	1374	2890	71.8%	5538
9	839.4	18.3%	2366	1933	42.2%	5450
10	1211	18.8%	1933	4655	72.2%	7434
11	1256	19.0%	2149	4793	72.3%	8201
12	964.9	18.8%	1651	3693	72.1%	6319
13	790.7	19.3%	1457	3008	73.6%	5544
14	874.7	19.0%	2465	1976	42.9%	5570
15	2225	18.8%	2175	8373	70.9%	8188
16	755.3	18.7%	2784	1603	39.6%	5908
17	294.9	20.4%	1472	691.9	47.9%	3453
18	565.7	19.3%	2824	1299	44.2%	6484
19	174.7	27.6%	761.0	403.0	63.6%	1755
20	601.3	19.5%	2881	1365	44.2%	6542
21	557.3	20.8%	2782	1267	47.3%	6325
22	130.9	18.6%	356.0	410.1	58.3%	1117
23	67.20	17.4%	335.0	192.0	49.7%	958.0
24	167.4	18.8%	255.0	651.6	73.3%	994.0
26	63.00	20.1%	335.0	148.6	47.4%	791.0
27	960.8	20.8%	12570	2659	57.6%	7240
29	615.9	20.9%	2708	1339	45.4%	5884
30	809.7	19.9%	2694	1731	42.6%	5759
31	1845	19.6%	2227	6738	71.7%	8132
32	308.7	20.5%	3082	809.4	53.6%	8080
33	73.30	13.5%	135.0	348.9	64.3%	643.0
34	1078	21.1%	1987	3825	74.7%	7049
35	433.8	22.3%	1907	945.7	48.5%	4157
36	1747	18.5%	2325	6903	72.9%	9188
37	1346	18.1%	2054	5160	69.3%	7875
38	1024	18.1%	2132	3939	69.6%	8205
39	158.9	17.9%	227.0	627.0	70.7%	894.0
40	81.90	11.8%	121.0	426.9	61.4%	629.0
42	89.30	18.3%	312.0	198.1	40.7%	693.0
43	89.10	18.8%	347.0	197.0	41.6%	767.0
44	174.5	17.5%	218.0	679.2	68.2%	848.0
45	75.70	19.0%	333.0	170.9	42.8%	751.0

46	51.70	11.9%	122.0	269.5	62.3%	636.0
47	283.3	20.1%	2340	733.0	52.1%	6056
48	426.8	21.3%	1876	1025	51.2%	4507
49	203.1	18.0%	253.0	795.3	70.6%	992.0
50	32.20	13.9%	119.0	93.80	40.6%	346.0
51	683.6	19.0%	1310	2609	72.4%	4996
52	76.60	17.6%	229.0	192.7	44.4%	577.0
53	167.3	17.5%	255.0	669.7	70.0%	1022
54	294.3	28.7%	1216	620.9	60.6%	2565
55	536.5	26.7%	1213	1717	85.5%	3880
56	724.4	20.2%	1710	2722	76.0%	6425
57	479.3	19.1%	2252	1086	43.3%	5101
58	113.7	18.2%	310.0	312.5	50.2%	851.0
59	95.80	15.9%	353.0	274.8	45.7%	1013
60	30.80	12.8%	127.0	88.70	37.0%	366.0
61	157.2	18.7%	649.0	395.2	47.1%	1632
62	586.1	18.8%	2160	1304	41.8%	4808
63	123.2	19.6%	335.0	344.1	54.7%	937.0
64	137.5	19.6%	392.0	386.7	55.1%	1103
65	933.4	22.7%	1479	3098	75.2%	4907
Total	35,090			112,000		
Mean	565.9	19.1%	1,625	1,806	57.0%	4,203
SD	496.5	2.90%	1,726	1,882	13.0%	2,883

Table 45. ACPF tool outputs for the large treatment wetlands after confirming the watershed dimensions with SWAT.

Wetland	Ponded Area (ha)	Total Area (ha)	Drainage Area (ha)
1	1.08	4.00	128.0
2	3.67	15.2	193.8
3	1.76	6.81	189.6
4	4.14	8.28	382.4
5	2.30	5.13	187.7
6	1.19	2.44	226.3
7	0.810	2.23	132.8
8	0.640	1.34	80.19
9	17.0	36.7	1204
10	10.3	36.5	523.4
Total	42.9	119	3,248
Mean	4.29	11.9	324.8
SD	5.30	13.6	335.8

Table 46. Calculations for nitrate-N reductions in the large wetlands.

Wetland	Mass Removed (kg)	Removal Efficiency (kg ha^{-1})
1	5081	1270
2	8619	567.8
3	6096	895.1
4	11410	1379
5	960.2	187.2
6	4863	1993
7	1784	800.0
8	345.6	257.9
9	20780	565.8
10	3425	94.00
Total	63,570	534.3
Mean	6,337	800.9
SD	6,123	602.3